# Mixed connective tissue disease:

## *Results from a nation-wide multicenter survey of Norwegian patients*

Thesis for the degree of philosophiae doctor (Ph.D.)

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## Paper I

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*Annals of the Rheumatic Diseases 2011;70(6): 1047-1051.* (1)

## Paper II

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Ragnar Gunnarsson, Trond Mogens Aaløkken, Øyvind Molberg, May Brit Lund, Georg Mynarek, Åse Stavland Lexberg, Kari Time, Alvilde Sofie Strand Dhainaut, Liv-Turid Bertelsen, Øyvind Palm, Karen Irgens, Andrea Becker-Merok, Jan Leidulf Nordeide, Villy Johnsen, Sonja Pedersen, Anne Prøven, Lamya Samir Noori Garabet, Jan Tore Gran.

*Annals of the Rheumatic Diseases 2012;71(12): 1966-72*. (2)

## Paper III

**Prevalence of pulmonary hypertension in an unselected mixed connective tissue disease cohort: Results of a nationwide, Norwegian cross-sectional multicenter study and review of current literature.**

Ragnar Gunnarsson, Arne K Andreassen, Øyvind Molberg, Åse Stavland Lexberg, Kari Time, Alvilde Sofie Strand Dhainaut, Liv-Turid Bertelsen, Øyvind Palm, Karen Irgens, Andrea Becker-Merok, Jan Leidulf Nordeide, Villy Johnsen, Sonja Pedersen, Anne Prøven, Lamya Samir Noori Garabet, Torhild Garen, Trond Mogens Aaløkken, Inge-Margrethe Gilboe, Jan Tore Gran

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# ABBREVIATIONS

| | |
|---|---|
| 6MWT | 6 minute walking distance test |
| ACR | American College of Rheumatology |
| AIP | acute interstitial pneumonia |
| ANA | anti-nuclear antibodies |
| anti-SSA/Ro | Sjögren's syndrome A antibodies |
| anti-SSB /La | Sjögren's syndrome B antibodies |
| ATS | American Thoracic Society |
| AZA | Azathioprine |
| BCDT | B cell depleting therapy |
| bFGF | basic fibroblast growth factor |
| BLyS | B lymphocyte stimulator |
| C | complement factor |
| CI | confidence interval |
| CFA | cryptogenic fibrosing alveolitis |
| CK | creatine kinase |
| COP | cryptogenic organizing pneumonia |
| CTD | connective-tissue diseases |
| CYC | Cyclophosphamide |
| DAD | diffuse alveolar damage |
| DIP | desquamative interstitial pneumonia |
| DIP | distal interphalangeal joint(s) |
| DLCO | diffusion capacity of carbon monoxide |
| DM | Dermatomyositis |
| dsDNA | double-stranded DNA |
| EMG | Electromyography |
| ERS | European Respiratory Society |
| ESC | European Society of Cardiology |
| FEV1 | forced expired volume in one second |
| FVC | forced vital capacity |
| GERD | Gastroesophageal reflux disease |
| GC | Glucocorticoids |
| GN | Glomerulonephritis |
| Hgb | Hemoglobin |
| HLA | human leukocyte antigen |
| HRCT | high resolution computed tomography |
| ICAM | intercellular adhesion molecule |
| ICD10 | International Classification of Diseases $10^{th}$ version |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IIM | idiopathic inflammatory myopathies |
| ILD | interstitial lung disease |
| IPF | idiopathic pulmonary fibrosis |

| | |
|---|---|
| IV | intravenous (injection) |
| jMCTD | juvenile mixed connective tissue disease |
| LIP | lymphoid interstitial pneumonia |
| MCTD | mixed connective tissue disease |
| MCP | metacarpophalangeal joint(s) |
| MHC | major histocompatibility complex |
| MMAs | myositis associated antibodies |
| MMF | mycophenolate mofetil |
| MMT | manual muscle test |
| MTX | Methotrexate |
| mPAP | mean pulmonary arterial pressure |
| MRI | magnetic resonance imaging |
| MSAs | myositis specific antibodies |
| NOSVAR | Norwegian Systemic Connective Tissue and Vasculitis Registry |
| NPSLE | neuropsychiatric lupus/SLE |
| NSD | Norwegian Social Science Data Services |
| NSIP | nonspecific interstitial pneumonia |
| NYHA | New York Heart Association |
| OP | organizing pneumonia |
| OUS | Oslo University Hospital |
| PAH | pulmonary arterial hypertension |
| PASP | pulmonary artery systolic pressure |
| PCWP | pulmonary capillary wedge pressure |
| PFT | pulmonary function tests |
| PH | pulmonary hypertension |
| PIP | proximal interphalangeal (joint(s)) |
| PM | Polymyositis |
| po | per oral |
| pro-BNP | N-terminal pro-brain natriuretic peptide |
| pSS | primary Sjögren syndrome |
| RA | rheumatoid arthritis |
| RAP | right atrial pressure |
| RB-ILD | respiratory bronchiolitis-associated interstitial lung disease |
| RCT | randomized controlled trials |
| REK | Regional Committee for Research Ethics |
| RNP | ribonuclear protein |
| RP | Raynaud's phenomenon |
| RTX | Rituximab |
| sc | Subcutaneous |
| SCLE | subacute cutaneous lupus erythematosus |
| SD | standard deviation |
| SLE | systemic lupus erythematosus |
| Sm | Smith antigen |
| SNP | single nucleotide polymorphism |
| snRNP | small nuclear ribonucleoprotein particle |
| SSc | systemic sclerosis |

| | |
|---|---|
| TRV | tricuspid regurgitation jet velocity |
| UCTD | undifferentiated connective-tissue disease |
| UIO | University of Oslo |
| UIP | usual interstitial pneumonia |
| VA | alveolar volume |
| VAS | visual analogue scale |
| VCAM | vascular cell adhesion molecule |
| VEGF | vascular endothelial growth factor |
| WHO | World Health Organization |

# SUMMARY

The Norwegian mixed connective tissue disease (MCTD) cohort study was the first nation-wide study of this rare autoimmune, systemic connective tissue disease (CTD), which was first defined only four decades ago (3).

This nation-wide, multicenter study involved the Departments of Rheumatology in Norway. The inclusion criteria were: age ≥ 18 years; a clinical diagnosis of MCTD; documented high titer serum anti-ribonucleoprotein antibody test; fulfillment of at least one of the three most used disease criteria for MCTD (4-6); and exclusion of other CTDs.

The study protocol included standardized questions about symptoms, clinical examinations, blood tests, high resolution computed tomography (HRCT), pulmonary function tests (PFT), echocardiography and in selected cases right heart catheterization (RHC).

The main results are presented in three papers. *Paper I* (1) showed a point prevalence of adult MCTD in Norway of 3.8 per 100,000 adults and the retrospective incidence estimate of adult-onset MCTD of 2.1 per million per year. *Paper II* (2) showed that pulmonary disease was frequent in the cohort, with as much as 52% of the patients having an abnormal HRCT. Lung fibrosis was identified in 35% of the patients, classified as minor (7%), moderate (9%) and severe (19%), with a clear preference for the lower parts of the lungs. After a mean of four years of observation, the presence of lung fibrosis was associated with increased mortality ($p < 0.05$). Preliminary analysis supported an association between lung fibrosis and esophageal dilatation evaluated by HRCT. *Paper III* showed that the overall prevalence of pulmonary hypertension (PH) in the cohort was 3.4%, a rate far lower than previously found in small scale studies (7-9). The mean follow-up was 5.6 years, from inclusion to the 1st of January 2012 when a total of 12 (8.2%) patients had died. Three of the five patients identified with PH died, two of whom had PH associated with severe ILD. The causes of death in the nine other deceased patients were ILD (n=2), coronary heart disease (n=2), cancer (n=4) and unknown (n=1).

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# 1 Mixed connective tissue disease

## 1.1 Introduction

The concept of mixed connective tissue disease (MCTD) as a separate immune-mediated connective tissue disorder was first introduced by Gordon C. Sharp and co-workers in 1972 (3). The concept of MCTD has been debated throughout the years as to whether the disease is a distinct identity or represents an overlap or early phase of other connective diseases (10-12). The concept of MCTD has now persisted for forty years.

The initial description (3) as a mild disease with favorable outcome and excellent effect of per oral prednisolone has, however, dramatically changed through the years and this is reflected in the four criteria sets for MCTD presented in 1986 (4-6) and 1991 (13).

MCTD is a disorder characterized by serum auto-antibodies directed against ribonucleoprotein (anti-RNP) (9, 14, 15) and distinct clinical features including: Raynaud's phenomenon (3, 8, 16, 17) , 'puffy hands', arthritis, pleuritis, pericarditis, myositis, esophageal dysmotility, pulmonary hypertension (PH) (7, 9, 15, 18-20) and interstitial lung disease (ILD) (21-27). Although some of the clinical features are also present in other connective tissue diseases (CTDs) such as systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM) and systemic sclerosis (SSc), the combination of clinical features with a positive test for anti-RNP antibodies appears to be unique for MCTD.

The research activity on MCTD has been low over the last decade compared to the other related CTDs. Our current knowledge of MCTD is mainly based on relatively small single-center hospital cohorts (7-9, 15, 19, 28, 29), the majority of results published two and three decades ago. There are mainly two reasons for this: the apparent rarity of the disease and the lack of internationally accepted disease/classification criteria of the disease.

## 1.2 Disease criteria

There are currently four criteria sets (Table 1) in use for diagnosis and classification of MCTD and they all are presented as diagnostic criteria. Three of these criteria sets (4-6) were first presented at an international conference on MCTD held in Japan in 1986 and the last criteria set was published in France in a rheumatology textbook in 1991 (13).

In 1996, Amigues and co-workers compared the sensitivity and specificity of the four criteria sets for MCTD (30). They concluded that Sharp's criteria (6) were most sensitive, but that the criteria of Alarcón-Segovia (4) and of Kahn (13) had better specificity. The only difference between those two is that Kahn's criteria set does not include *acrosclerosis* as a clinical criterion as does Alarcón-Segovia. Sharp's criteria set is generally considered to be too complicated to be used in clinical practice or in clinical studies. No comparison between the diagnostic MCTD criteria sets has been done in an epidemiological setting.

**Table 1. Overview of disease criteria of mixed connective tissue disease (I-IV)**.

**Diagnostic criteria for MCTD, by Gordon C. Sharp [6]**

| | |
|---|---|
| **Definite** | 4 major criteria, and anti-U1 RNP positive, with anti-ENA ≥1:4000. Exclusion: anti-Sm |
| **Probable** | 3 major criteria and anti-U1 RNP positive with anti ENA ≥1.1000<br>2 major criteria (include 1 or more from #1, #2, #3 and 2 minor criteria, and anti-U1 RNP positive with anti- ENA ≥1.1000) |
| **Possible** | 3 major criteria<br>2 major criteria and anti-U1 RNP positive with anti-ENA ≥1:100<br>1 major and 3 minor, and anti-U1 RNP positive with anti-ENA ≥1:100 |

| **Major criteria** |
|---|
| 1. Myositis, severe |
| 2. Pulmonary involvement |
| a. CO diffusing capacity < 70% of normal |
| b. Pulmonary arterial hypertension |
| c. Proliferative vascular lesions in lung biopsy |
| 3. Raynaud's phenomenon, or esophageal hypomotility |
| 4. Swollen hands observed or sclerordactyly |
| 5. Highest observed anti-ENA≥1:10,000 and anti U1 RNP positive and anti-Sm negative |

| **Minor criteria** |
|---|
| 1. Alopecia |
| 2. Leukopenia <4,000 WBC/$mm^3$ |
| 3. Anemia ≤10.0 g/dL females, <12.0 g/dL males |
| 4. Pleuritis |
| 5. Pericarditis |
| 6. Arthritis |
| 7. Trigeminal neuropathy |
| 8. Malar rash |
| 9. Thrombocytopenia (<100,000/$mm^3$) |
| 10. Myositis, mild |
| 11. Swollen hands or history of swollen hand |

## II. Classification and diagnostic criteria for MCTD, by Donato Alarcón-Segovia and Miguel Villareal. [4]

**Serological criteria:** *(obligatory)*
Positive anti-RNP at a hemaglutination titer ≥ 1:1600

**Clinical criteria:** *( ≥ 3**)*
1. Edema of the hands
2. Synovitis
3. Myositis (laboratory or biopsy proven)
4. Raynaud's phenomenon (2 or 3 color phase)
5. Acrosclerosis (with or without proximal scleroderma)

** *The association of edema of the hands, Raynaud's phenomenon and acrosclerosis requires the addition of at least one of the other two criteria.*

## III. Preliminary diagnostic criteria for classification of MCTD, by Reiji Kasukawa et al. [5]

**I. Common symptoms** *(obligatory ≥ 1)*
1. Raynaud's phenomenon
2. Swollen fingers or hands

**II. Anti-nRNP antibody** *(obligatory)*

**III. Mixed findings***: (obligatory ≥1 findings in two of disease categories; A, B, or C)*

**A. SLE-like findings**
1. Polyarthritis
2. Lymphadenopathy
3. Facial erythema
4. Pericarditis or pleuritis
5. Leucocytopenia (> 4,000/$mm^3$) or thrombocytopenia (>100,000/$mm^3$)

**B. Systemic sclerosis-like findings**
1. Sclerodactyly
2. Pulmonary fibrosis, restrictive change of lung (%VC < 80%) or reduced diffusion capacity (DLCO < 70%)
3. Hypomotility or dilatation of esophagus

**C. PM-like findings**
1. Muscle weakness
2. Increased serum level of myogenic enzymes (CPK)
3. Myogenic pattern in EMG

## IV. Diagnostic criteria for MCTD, by M.F. Kahn and T. Appelboom. [13]

**Serological criteria:** *(obligatory)*
Positive anti-RNP (verified by immunodiffusion or immunoblot (U1 68kd) anti-RNP ≥ 1/2000)

**Clinical criteria***: (≥3)*
1. Raynaud's phenomenon *(obligatory)*
2. Synovitis
3. Myositis
4. Puffy hands

## 1.3 Epidemiology

In spite of the fact that four decades have passed since Sharp and co-workers first introduced the concept of MCTD (3), there have been no published prevalence or incidence data on adult MCTD based on one or more of the four disease criteria (31). MCTD may start in childhood or at a juvenile age (jMCTD), commonly defined as before 16 years of age (32-34), but in some instances as up to 18 years of age (35).

A population-based epidemiological inquiry from Finland in 1990 looked at cases identified by nationwide sickness insurance schemes to receive specially reimbursed medication and found a point prevalence of MCTD as low as 0.8 /100,000 adults. These calculations were based on a clinical diagnosis of MCTD and none of the four known disease criteria were used (36). Another report, also from Finland, suggested that the incidence of MCTD in children is 0.1 patients per million per year (37).

MCTD seems to affect women more frequently than men (8, 9, 17) and this observation is referred to in the several small cohort studies. A follow-up study by Bennett and O'Connell (19) found a female to male ratio of 19. A long time follow-up study of a cohort of 47 patients by Burdt et al. (8) found a female to male ratio of 10.8. Similar results were found in two previous cohorts from the same institution (7, 9). The lowest ratio has been found in a cohort study from Sweden by Lundberg and Hedfors (17) with a female to male ratio at 4.7.
There are reports of MCTD in all races. Studies have emerged from Japan, Europe including Scandinavia, the US including patients also of African-American ancestry, South America (27), the Middle East (38) and India (39).

One important issue regarding MCTD is the phenotypic stability of the disease. Earlier notions of MCTD being a preclinical form of other CTDs and that the patients would subsequently develop more classical forms of SLE, SSc or PM/DM, do not seem to apply in long-term follow-up cohort studies spanning up to three decades (8). Several heterogeneous cohort studies with different definitions of the disease have shown mixed results (8, 28, 40-42).

## 1.4 Genetics, autoantibodies and pathogenesis

The etiology of MCTD remains obscure. Environmental factors such as viral and/or other infections, factors in the food, drugs, biological agents, radiation and several other possible environmental factors in addition to host-related genetic, hormone related and complex interactions have all been suggested as causative factors (43-47).

### 1.4.1 Genetics

The best way to estimate the relative impact of environmental and genetic influences in humans is by comparing monozygotic (MZ) and dizygotic (DZ) twins. Regrettably no twin studies have been performed on MCTD and currently only one case of monozygotic twins having MCTD (48) has been published. A comparison with related diseases such as SLE and SSc can yield information In SLE the concordance rate between monozygotic twins is between 24% to 58% and between 2% to 5% among dizygotic twins (49, 50). As to systemic sclerosis the concordance was found to be similar in MZ and DZ twins with an overall low concordance of 4.7% after analyzing data from 42 twins in which at least one of them had been diagnosed with SSc and finding a concordance for SSc in just two (51).

Several small scale cohort studies have found a genetic association with HLA-DR4 (8, 41, 52-59) by comparing MCTD patients to either healthy controls or patients with SLE and/or SSc. In one study an association with HLA-DR1was found, when comparing with SLE patients (53). In another study an association with HLA-DR2 (41) appeared by comparing patients with SLE and/or SSc. No association has been reported, however, between MCTD and HLA-DR3 bearing haplotypes strongly associated with SLE, or with HLA-DR5 bearing haplotypes associated with SSc (8).

The main conclusion from current knowledge of MCTD implies that the HLA association is different from both SLE and SSc.

### 1.4.2 The anti-RNP antibody

The characteristic antibody in MCTD is directed against the U1-snRNP complex that plays an essential role by removing introns from transcribed pre-mRNA (hnRNA) segments in a

process that is generally referred to as splicing. It is still unclear which part of the snRNP complexes plays a role in autoimmune processes or if they initiate the autoimmune processes and/or drive them further and/or if they are mainly bystanders. The U1-RNA, as one of five classes (U1-, U2, U4, U5 and U6-RNA) found in humans, forms the structural backbone of 165 nucleotides which forms a four stem loop when it folds three dimensionally. There are three highly specific U1-RNA proteins, the U1-70K (also called U1-70, U1-70kD and U1-68K), the 33kD (U1-A) and the 22kD (U1-C). In addition there are splicing factors (SR proteins) and the Sm (Smith) proteins connected to the U1-RNA (Figure 1) (43). Antibodies to U1-70K snRNP are characteristic for MCTD (44, 60). Burdt and coworkers (8) observed in an long-term cohort study of 47 patients, an "…orderly progression of autoantibody reactivity against snRNP polypeptides, with intramolecular epitope spreading followed, in sequence, by 'epitope contraction' and subsequent disappearance of snRNP autoantibodies during disease remission" (8).

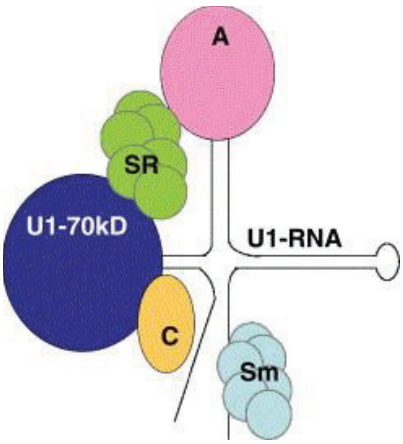



**Figure 1. The U1- ribonucleoprotein complex**
The U1-RNA has double-stranded secondary structure. The U1-70kD protein binds specifically to the first stem-loop; the U1-A protein binds similarly to the second stem-loop. The Sm proteins bind to an area on a fourth stem-loop. The SR and U1-C proteins participate in protein–protein interactions with other members of the U1-RNA. (Adapted from Greidinger et al., Rheumatic Diseases Clinics of North America, 2005) (43)

Antibodies against nuclear components are found in several autoimmune connective tissue diseases, including antibodies against the RNA-associated molecules, the small ribonucleoprotein particles (snRNPs), signal recognition particle (SRP), tRNA synthetase antibodies (anti-histidyl (Jo-1)/ threonyl (PL-7)/ alanyl (PL-12)/ isoleucyl (OJ)/ glycyl (EJ)/ asparaginyl (KS) tRNA synthetase ), anti-RNA polymerases (I, II and III), SS-A/Ro and SS-B/La.

In the frequently cited study of Arbuckle et al. (61), the authors had access to the US military biobank containing annual serial blood samples from military personnel who developed lupus-like disease. By analyzing serial blood samples from these patients several years before the clinical disease the interval between the first detection of antinuclear, antiphospholipid, anti-SS-A/Ro, and anti-SS-B/La antibodies was apparent at a mean of 3.4 years before SLE disease manifestations. Anti–double-stranded DNA (dsDNA) antibodies were first detected a mean of 2.2 years before diagnosis, while anti-Sm and anti-RNP antibodies appeared latest, with a mean of 1.2 years before the start of SLE (61). In the case of SLE patients, the appearance of anti-RNP antibodies was seen late in the preclinical phase of the disease. Whether this is the case also for MCTD patients is currently not known as such studies have not yet been conducted on MCTD.

### 1.4.3 Pathogenesis

In a mouse model (62), a production of anti-RNP 70K antibodies in the mice led to the involvement of perivascular and interstitial infiltrates in their lungs, a clinical picture similar to human MCTD. This mouse model was the knockout C57BL/6-derived mice, transgenic for human HLA-DR4, being immunized with U1-70K fusion protein along with either complete Freund's adjuvant (CFA) or U1 RNA. In the same study, using the same setting, but by changing the innate immune signaling system by analyzing for the Toll-like receptor 3 (TLR-3) deficient mice, showed that the mice then did not develop MCTD-like pulmonary disease, but rather developed SLE-like disease with glomerulonephritis (62). This clearly implies a pathogenic role for anti-U1-70k antibodies, at least in a murine animal model.

Recent work by Distler et al. (63) links dysregulation of factors modulating angiogenic responses with the pathogenesis of MCTD. They reported increased serum levels of the angiogenic vascular endothelial growth factor (VEGF) in patients with MCTD and SSc compared to healthy controls. In MCTD patients there was also a selective up-regulation of endostatin, a factor that exerts potent anti-angiogenic effects and inhibits endothelial cell migration and proliferation. This was most apparent in a subset of MCTD patients having complications such as pulmonary arterial hypertension, acrosclerosis and active myositis. In another study, in mice, modestly increased levels of VEGF resulted in microangiopathy that closely resembled findings in human patients with SSc and MCTD (64).

The main conclusion is that anti-RNP antibodies interplaying with the innate immunity may have a pathogenic role in MCTD, at least in a murine model. And the dysregulation of the angiogenic response may be at least one of the mechanisms that drives the disease into different clinical subsets.

## 1.5 Key clinical features of MCTD

In the following section, we will review the key clinical features of MCTD. The current knowledge of the disease has mainly been based on tertiary hospital cohorts (Table 2).

**Table 2: Cohort studies of MCTD and/or anti-RNP positive patients.**

| Study | Year | Country | No. pts. MCTD (RNP+) | F:M ratio | Criteria | Follow-up [years] | |
|---|---|---|---|---|---|---|---|
| Bennet et al. (19) | 1980 | USA | 20 | 9.0 | N/A | N/A | Single tertiary center review of pts. seen 1972-1976 |
| Nimelstein et al. (28) | 1980 | USA | 22 | 4.3 | N/A | N/A | Review of the original 22 of the 25 patients from Sharp`s original article (3). |
| Grant et al. (65) | 1981 | USA | (23) | 2.8 | N/A | N/A | Review of medical files of 23 pts. with high anti-RNP. |
| Alpert et al. (7) | 1983 | USA | 38 | 11.7 | N/A | N/A | Tertiary single center study regarding cardio-vascular manifestations. |
| Sullivan et al. (9) | 1984 | USA | 34 | 10.3 | N/A | 6.3 | Tertiary single center cohort follow-up |
| Kitridou et al. (66) | 1986 | USA | 30 | 14.0 | N/A | 9.4 [±1.1] | Single center cohort including pts with low titer anti-DNA (in 3), anti-Sm (in 3) and low complements (in 12). |
| Black et al. (52) | 1988 | UK | ? (46) | 10.5 | N/A | N/A | 46 anti-RNP positive pts, 9/46 had SLE, 4/46 had SSc and 3/46 had both SLE and SSc at inclusion. |
| Lundberg et al. (17) | 1991 | Sweden | 17 (32) | 4.7 | A-S | 5.4 [2-10] | Single center observational study of pts. w/ high anti-RNP. |
| van der Hogen et al. (42) | 1994 | Nether-lands | 33 (46) | (2.5) | N/A | 15±6 | Single center, retrospective analysis of 46 anti-RNP pos. pts. 10/46 had SLE, and 33 (72%) were classified as MCTD. |
| Gendi et al. (41) | 1995 | UK | ? (39) | 8.8 | N/A | N/A | Follow-up of the study by Black et al. from 1988 (52). 39 out of 46 were accessible. |
| Burdt et. al. (8) | 1999 | USA | 47 | 10.8 | KC | 15 [3-29] | Follow-up cohort, single tertiary center, 23% had juvenile onset disease. |
| Rayes et al. (38) | 2002 | Saudi Arabia | 18 | 2.5 | A-S | 5 [1-17] | Single center retrospective study of MCTD pts. from 1982 to 1999 |
| Lawrence et al. (39) | 2007 | India | 16 | 15.0 | SC/A-S /KC | 1 [0-14.3] | Single center retrospective analysis of MCTD pts. |
| Cappelli et al. (40) | 2011 | Europe‡ | 161 | 11 | SC/A-S /KC | 7.8 | Retrospective analysis of journal information from 15 tertiary centers in 8 countries. ‡ |

A-S = Alarcón-Segovia's criteria (4), KC = Kasukawa's criteria (5), SC = Sharp's criteria(6), USA = United States of America, UK = United Kingdom, pts. = patients, RHC = right heart catheterization,
‡ Italy, Croatia, Romania, Serbia, France, Hungary, Germany, Russia

### 1.5.1 Raynaud's phenomenon and "puffy hands"

**Raynaud's phenomenon**

Maurice Raynaud, a French doctor, recognized in 1862 that some people who were exposed to cold temperatures had transient digital ischemia (67). Raynaud's phenomenon (RP) is commonly defined as episodic ischemic attacks of digital vasospasm triggered by exposure to cold, stress or trauma. RP usually involves the fingers and the toes, but frequently also involves the ear lobes and the nose. In addition, RP may also affect the tongue (68) and the nipples (69). RP leads to typical three or dual color changes. The first phase *pallor*, is a consequence of exaggerated vasoconstriction. The second phase, *cyanosis*, is due to reduced venous flow and desaturation of residual blood, and finally the third phase, *rubor*, is due to reactive hyperemia (16, 67).

The diagnosis of RP is mainly based on history. Photographs during an attack may confirm the diagnosis. Provocative tests are not commonly considered necessary to make a definitive diagnosis. Special laboratory-based tests are complex and generally not used clinically to distinguish patients with RP from cold-sensitive persons that normally does not involve three phasic color changes (67).

RP is normally divided into primary and secondary forms. Primary RP is caused by benign functional vascular defects and secondary RP is associated with underlying chemical and/or structural changes in the vessel walls and in general presents with more severe symptoms.

In the absence of an underlying systemic disorder, RP is defined as primary and more often affects younger individuals and women more than men. There seems to be a huge geographical difference in the prevalence of primary RP. One study comparing patients in South Carolina, US, and Savoie, France, estimated prevalences of 5.0% and 16.8%, respectively (70). Patients with primary RP are mainly treated with lifestyle modifications of keeping warm and cessation of smoking

Secondary RP is associated with systemic diseases, most often connective tissue diseases. The highest prevalence is found in patients with SSc and MCTD (16). Microscopy of the nail fold capillaries (71) appears to be a valuable tool to differentiate between primary and secondary RP. There are no structural changes in nail folds of patients with primary RP, whereas

patients with SSc (72), MCTD and DM may have characteristic structural changes (73, 74). RP is one of the predominant symptoms of MCTD (3) and is an integral part of all the four proposed and preliminary criteria of MCTD (4-6, 13).

The follow-up cohort study of Lundberg and Hedfors showed that in the course of their study 94% of the MCTD patients had RP (17). Burdt and coworkers (8) found respectively 74 % and 96% with RP on initial presentation and as cumulative findings, and RP was found 91% in the earlier study of Sullivan et al. (9).

To summarize, RP was included in all the four disease criteria for MCTD. RP was found in the majority of patients during the course of the disease.

**"Puffy" or swollen hands**

'Puffy' or 'swollen' hands indicate symmetrical inflammation of the fingers and hands and is one of the most common manifestations in MCTD. 'Puffy/swollen' hands are included in all of the four current disease criteria sets (4-6, 13). 'Swollen' or 'puffy' hands have been reported for between 60 and 94% in MCTD cohorts (17, 66). Swollen hands were reported in 60% of a cohort consisting of patients from South California (Table 2) (66). On the other hand in a cohort study from Sweden, which included Caucasians, 94% of the 17 MCTD patients had 'puffy hands' (17). In a long-term follow-up cohort studied by Burdt et al. (8), swollen hands presented initially in about half part of the cohort (45%) and were seen in a total of two thirds (66%) of the patients during the course of the disease. The pathological basis of 'puffy hands' is not clear, it is either tenosynovitis and/or endothelial cell and blood-vessel pathology (75).

### 1.5.2 Myositis

Myositis is included as one of the major criteria in all the four criteria sets for MCTD (4-6, 13), but only a very few studies have examined this disease manifestation (76). The available data do, however, suggest that the myositis in MCTD is less severe than in idiopathic inflammatory myositis. Various studies show that myositis was found in the course of the MCTD in 35-79% of patients (3, 8, 9, 17, 19, 66). This was based on various objective criteria, such as muscle biopsies, electromyographies (EMG) and/or high creatine kinase (CK) as markers of muscle involvement often associated with muscle weakness and/or myalgia.

Currently there are no studies using magnetic resonance imaging (MRI) as a marker of myositis in MCTD.

Myositis is rarely a presenting symptom of MCTD and only found in 2% of those studied a long-term follow-up cohort study (8) as opposed to 51% in the course of the disease and at time of diagnosis in 28%. There are no studies presenting a detailed description of muscle involvement in MCTD; however it appears that proximal musculature in the lower extremities is most commonly affected, similar to muscle involvement associated with PM/DM is most common (76). There are also reports of heart muscle involvement (77), but clinically this does not seem to be common.

In a large European cohort of inflammatory myositis (78), autoantibodies to anti-U1snRNP antibodies were detectable in 6% (25/417). When reviewing the histological classification of myositis, anti-RNP antibodies were found in 9% (17/198) of the patients classified as PM, in 4% (7/181) in those classified as DM, and additionally in 3% (1/38) of those classified as inclusion body myositis (IBM).

Patients with MCTD started with a lower steroid dose compared to the patients with PM and DM and succeeded in reducing it earlier and seemed to have a better prognosis regarding muscle involvement (79).

### 1.5.3 Arthritis and arthralgia

Joint symptoms are commonly associated with MCTD in most studies. Arthritis is one of eleven minor criteria in the criteria set of Sharp (6) and is one of the clinical criteria of Alarcón-Segovia (4) and of Kahn (13) and polyarthritis is one of the 'SLE-like findings' in the criteria set of Kasukawa et al. (5).

**Arthralgia**

Arthralgia or joint pain was in an early study of Ramos-Niembro et al. (80), found to be the first symptom of the disease in 50% (14/28) and one of the two first symptoms of the disease in 86% of cases (24/28). The arthralgia was reported to be polyarticular in 82% (23/28), pauciarticular in 14% (4/28) and not present in 4% of cases (1/28). In Lundberg's study (17) all the 17 patients with MCTD had arthralgia in the course of the disease.

**Arthritis**

The arthritis associated with MCTD is commonly polyarthritis. In the cohort study of Sullivan and al. (9) 85% had arthritis, whereas 97% (29/30) had clinical arthritis in the course of the disease in the study by Kitridou and coworkers (66). A total of 17% (5/30) had an erosive and deforming arthritis. In the longitudinal clinical follow-up cohort of 47 patients presented by Burdt and coworkers (8), arthralgia and/or arthritis was one of the presenting symptoms in 68% and was seen in the course of the disease in a total of 96%. In Lundberg's study 88% (15/17) had clinical arthritis (17) where one third had polyarthritis resembling RA with a slow progression of marginal discrete erosions mainly of the bones of the hands was the most characteristic findings. In their study, non-deforming polyarthritis affected mainly the wrists and the ankles (15). In the study of Ramos-Niembro (80) most of the patients had clinical arthritis or 89% (25/28), all with arthritis in the MCP joints (25/28), and 43% of those had arthritis in the PIP joints of the hands. The development of arthritis was found in the knees (75%), wrists (64%), elbows (46%) and ankles (36%). Only one patient (1/28) had arthritis in the temporomandibular joint, and none had documented arthritis in the hips, the DIP joints of the hands or in the shoulders, though 19/28 joint pain located to shoulders. It is, however, important to mention that the study did not include MRI and/or ultrasound in the diagnosis of arthritis that could have increased the prevalence of arthritis. The x-ray findings were the classical findings of joint space narrowing; erosions both cystic and marginal; and general osteoporosis of the hands was considered present in 82% of the patients. In the study of Bennett (81) all had arthritis and/or arthralgia. A total of 85% (17/20) of the patients had evident clinical arthritis most frequently involving MCP and PIP joints of the hands, wrists and MTP joints, and none had affection of hips, temporomandibular joints, SI-joints, sterno-clavicular joints nor acromioclavicular joints. A total of 60% (12/20) had erosions of the bones in the hands confirmed by x-ray, showing marginal and discrete findings in most patients. Joint deformities were present in 30%, consisting of ulnar deviation (3/20), destructive arthritis (2/30) and 'swan neck' deformities (1/20) (81).

Extensive x-ray analysis of serial fine-detail radiographs (82) showed that 59% (10/17) of the patients had definite (grade 2 or 3) periarticular osteopenia and 47% (8/17) had diffuse osteopenia in the hand as evaluated with x-ray. Joint-space narrowing was noted in 41% (7/17) and 53% (9/17) had erosive lesions, thereof three of the total five patients that also were seropositive for rheumatoid factor and included in the study. There are isolated reports of MCTD patients demonstrating joint changes in MRI (82, 83). The arthritis diagnoses in

current MCTD studies were solely based on clinical findings and erosions and/or joint space narrowing based on estimations of conventional X-ray investigations.
The main conclusion is that arthralgia (85-100%) and arthritis (85-97%) are found in the majority of MCTD patients in the course of the disease the deforming arthritis seem to be rare. But as stressed, the basis for our knowledge, are few small and relative old studies not using the latest diagnostic modalities.

### 1.5.4 Skin manifestations and alopecia

Skin manifestations are not included either by Alarcón-Segovia (4) or in Kahn's criteria (13) for MCTD, but are included as two of the eleven 'minor criteria´ in Sharp's criteria (6) as 'alopecia' and 'malar rash' and as 'facial erythema' as one of the five of the 'SLE-like findings' in the Kasukawa criteria set (5).

Erythematous skin rash was present on the initial presentation of the disease in 13% and cumulative in 53% in the course of the disease in the long-term follow-up cohort study of Burdt (8). In the study of Sullivan (9) 29% had malar rash and 41% had alopecia. A similar number of hair loss cases was reported in the study of Bennett et al. (19). A skin rash was found in a total of 47% of the 17 MCTD patients in the study of Lundberg and Hedfors (17).

There are case reports of MCTD patients having livedoid vasculitis (84). Epidermal pathology of MCTD mimicked that of subacute cutaneous lupus erythematosus (SCLE) but a concomitant vasculopathy parallel to that seen in skin lesions of dermatomyositis distinguished the dermatopathology of MCTD from that of SCLE in a study of the cutaneous eruptions of eight patients with MCTD (85).

Hair loss associated with MCTD is seemingly not as common as that associated with SLE. Skin biopsies encountered prospectively from 20 patients, including four patients with MCTD, demonstrated neutrophilic or suppurative granulomatous folliculitis as a potential cause (86).

### 1.5.5 Gastrointestinal involvement

Gastrointestinal symptoms such as esophageal dysmotility and hypomotility are an integral part of MCTD and included as part of one of the five 'major criteria' by Sharp (6) and as part

‘mixed finding’ as ‘systemic sclerosis-like finding’ by Kasukawa (5). However, gastrointestinal involvement is not included either in the criteria sets of Alarcón-Segovia (4) and of Kahn (13).

There are various gastrointestinal involvements in MCTD. There seem to be similarities between the gastrointestinal manifestations of MCTD and that of SSc. Esophageal dysmotility seems to be common. In one study, 88% of the patients with MCTD had an abnormal manometry (87, 88). A total of 18% (3/17) of the MCTD patient in Swedish cohort study had dysmotility of the esophagus (17), compared to 66% in the study of Burdt (8) and 57% of the patients from Sharp’s initial MCTD patients (3, 28).

There are isolated reports of MCTD with autoimmune hepatitis (89-91), primary biliary cirrhosis (92), pancreatitis and portal hypertension (93); these manifestations, however, seem to occur infrequently. There are a few case reports of patients with MCTD with protein loosing enteropathy (94, 95) and pneumatosis intestinalis (96, 97), a rare disease manifestation with gas cysts in the bowel wall. One single case of angiodysplasia of the gastric antrum with a pattern of tortuous vessels located along the longitudinal folds of the stomach with endoscopic appearance similar to watermelon, commonly named ‘watermelon stomach,’ has been presented (98), whereas such manifestations seem to be more frequently observed in SSc.

In conclusion, gastrointestinal involvement, other than dysmotility of the esophagus, seems to be rarely associated with MCTD.

### 1.5.6 Hematological manifestations

Leucopenia and/or thrombocytopenia are included in the criteria sets by Sharp (6) and Kasukawa and coworkers (5) but not in the criteria sets by Alarcón-Segovia et al. (4) nor in those of Kahn and Appelboom (13).

In the long-term follow-up cohort study by Burdt (8) 53% had leucopenia and/or lymphopenia in the course of the disease and 11% as an initial presentation. In the early review of the initial patients in Sharp’s cohort (28), leucopenia was present in 41% (9/22) and anemia in 55% (12/22). In the Lundberg’s study (17), 65% (11/17) had anemia, 24% (4/17) had leukopenia and 6% (1/17) thrombocytopenia. In the early study of Bennet et al. (19) of twenty patients

75% had leucopenia (defined as white blood cell count < 4,000/$mm^3$) and 75% had anemia (defined as Hgb < 11.5 g/dl in females or < 12.5 g/dl males).

The main conclusion is that anemia and leucopenia are common findings during the disease course of MCTD, whereas thrombocytopenia seems to be more infrequent.

### 1.5.7 Neurologic manifestations

Neurological manifestations are included only in Sharp's criteria set (6) where trigeminal neuropathy is one of the eleven 'minor criteria'.

The most common neurological manifestation seems to be trigeminal neuropathy (28) followed by other peripheral neuropathies. There are case reports of aseptic meningitis (28), transverse myelitis and organic brain syndrome. Neuropsychiatric manifestations commonly associated with SLE and other central nervous complications are considered to be rare manifestations in MCTD. This was also confirmed in a recent article by Nowicka-Sauer and coworkers (99), where the main conclusion was that the majority of MCTD patients rarely presented severe impairment of cognitive functions (99).

A Hungarian study assessing prevalence of sensory-neural hearing loss in MCTD showed a more than double increase in the frequency of sensory-neural hearing loss as registered by audiograms in patients with MCTD compared with healthy controls (100). There was no correlation between age and disease duration, but an association was found with autoantibodies (anti-U1RNP, anti-endothelial cell antibodies and IgG type anti-cardiolipin antibodies).

Thus, peripheral nervous manifestations may occasionally be observed in MCTD while central nervous manifestations seem extremely rare.

### 1.5.8 Renal involvement

Renal involvement is not included in any of the four criteria of MCTD (4-6, 13). Kidney disease was initially considered a rare manifestation of MCTD, mostly being subclinical with a benign disease course (101).

Biopsy verified renal disease was found in 11% (5/47) in the course of the disease in the long-term cohort study by Burdt (8). Kidney biopsies showed changes similar to Lupus WHO class

III glomerulonephritis (GN) in three patients, whereas one patient also had Lupus WHO class V GN changes. Additionally two patients had Lupus WHO class IV GN changes. In a review of Nimelstein et al. (28) of the original Sharp's publication (3) 5% (1/22) had renal disease and in the study of Bennet (19, 102) 20% (4/20) of the patients had biopsy-proven immune complex nephropathy histologically classified as membranous GN in three patients. Similar findings were also confirmed by a cohort study by Sullivan and coworkers (9) where renal abnormalities were detected in 26%, but 18% had clinically evident nephritis without any of the patients developing significant renal dysfunction.

In the Lundberg study (17), 29% (5/17) had urinary findings, but biopsy verified glomerulonephritis was only confirmed in one patient associated with a decreasing anti-RNP titer and a rising anti-DNA titer. Review of five Japanese MCTD patients with renal involvement (103) showed clinical renal involvement in just one of the five patients, histologically classified as diffuse mesangial proliferative GN in 60% (3/5) and membranous glomerulonephritis in 40% (2/5).

The most common form of renal involvement seems to be an immune complex nephropathy (66) histologically classified as membranous GN (66, 103). Other reported forms of renal involvement are membranoproliferative GN, mesangial proliferative GN (103) and focal and segmental glomerulosclerosis. In addition, there are reports of minimal change disease focal proliferative crescentic nephritis with necrotizing arteritis (66, 104). There are few reports of patients with MCTD developing a rapid renal crisis with hypertension, similar to a scleroderma renal crisis (105, 106). One of these cases reported severe renal intimal hyperplasia, mostly in the interlobular arteries with mild glomerular changes and with collapsed glomeruli were seen with histopathological features resembling those seen in a scleroderma renal crisis (105).

In conclusion, renal involvement can affect about one fifth (20%) of MCTD patients in the course of the disease, but kidney involvement is mostly subclinical with good prognosis.

### 1.5.9 Cardiac manifestations

Pulmonary arterial hypertension is included as one of the five 'major criteria' and pericarditis is one of the 'minor criteria' of Sharp (6). Pericarditis is included as one of the five 'SLE-like findings' in the Kasukawa criteria (5) and pulmonary hypertension indirectly could be

considered when the diffusion capacity of carbon monoxide ($D_LCO$) is below 70% of predicted value as referred to in the 'Systemic sclerosis-like findings'. Cardiac manifestations are not included in neither the Alarcón-Segovia (4) nor in the Kahn's criteria (13). Differences in the criteria sets may select different subset of patients which have to be born in mind when reviewing and planning studies..

In a single center hospital study of 20 patients, pericarditis was found in 20% (4/20), of whom one had pericardial disease documented by echocardiography while in the other three patients, the diagnosis of pericarditis was based on clinical findings (19). The frequency of cardiac involvement in MCTD is still discussed. Studies vary in the length time until follow-up time, in the various degrees of objective findings and finally in the definition of electrocardiography, echocardiography, right heart catheterization or other special investigations. In a 2005 review article of cardiac manifestations of MCTD (18), the prevalence of cardiac involvement was found to be between 11% (17) and 85% (7). With the exception of pulmonary hypertension, which will be reviewed in detail later, the cardiac manifestations in MCTD seem to be mild in most cases. The most frequent forms of cardiac manifestations seem to be pericarditis and mitral valve prolapse. There are reports of myocarditis coinciding with myositis (107) and a case of hypertrophic obstructive cardiomyopathy confirmed by myocardial biopsy (108).

### 1.5.10 Pulmonary hypertension

Pulmonary hypertension (PH) is increased blood pressure in the pulmonary vasculature leading to hypoxemia. The initial symptoms are shortness of breath with decreased exercise tolerance that, untreated, may progress to severe hypoxemia with right heart failure. Symptoms of PH may develop very gradually and there are both patient and physician delays in diagnosis. Other common symptoms of PH are fatigue, non-productive cough and syncope. The patients may have signs of right heart failure with peripheral edema, hemoptysis and/or angina pectoris. The main clinical findings can be a loud second heart sound (S2) from the closure of the pulmonic valve and parasternal heave. If the patients evolve right ventricular failure (*cor pulmonale),* there are clinical signs as: jugular venous distension, hepatojugular reflux, ankle edema, enlarged liver, ascites and pleural effusion. The patients may also have clubbing as a consequence of chronic hypoxemia.

The electrocardiogram (ECG) may demonstrate signs of right ventricular hypertrophy, such as tall right precordial R waves, right axis deviation and right ventricular strain with right bundle branch block, a high P wave, an R/S ratio >1 in lead V1. ECG findings are seen most often in patients with advanced PH (109, 110). Enlargement of the central pulmonary arteries on conventional chest radiography is better viewed with computed tomography (CT) of the thorax (111, 112). Predicted carbon monoxide diffusion capacity ($D_LCO$) is usually markedly reduced in patients with PH (113).
Estimation of the dyspnea is commonly done by either the New York Heart Association functional classification (114) (Table 3) originally adopted for use in heart failure patients and the related WHO 1998 Functional classification of pulmonary hypertension (115) (Table 4). Estimation and treatment effect are evaluated with a standardized 6 minute walking distance test (6MWT) (116).

PH is an important cause of morbidity and mortality in systemic connective tissue diseases, particularly in systemic sclerosis. According to the updated clinical classification of PH (the Dana Point classification) (117) from 2008, the patients with connective tissue disease and PH mainly belong to two main categories: They have either pulmonary arterial hypertension (PAH) without pulmonary disease (Dana Point Group 1.4.1) or they have PH associated with interstitial lung disease (PH-ILD) (Dana Point Group 3.2). Some cases of PH may be due to chronic thromboembolic pulmonary disease (CTEPH) (Dana Point Group 4). Other causes of PH in MCTD seem to be extremely rare, such as pulmonary veno-occlusive disease (PVOD) (118) and/or pulmonary capillary hemangiomatosis (PCH) (Dana Point Group 1') but currently there is only one reported case of PVOD associated MCTD (119). In the few, but frequently cited studies on PH in MCTD (7-9), PAH and PH-ILD are commonly used as interchangeable terms.

The serum biomarker, N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) has prognostic value for PH (120). NT-proBNP is a natriuretic peptide secreted mainly by the ventricles in response to increased mechanical stretch and pressure (121, 122), and an increased NT-proPNP level has been shown to have a negative prognostic value in various forms of PH. Data from SSc showed a correlation between increased NT-proBNP levels and PH (123), but no such association has been studied in MCTD and PH.

The pulmonary artery systolic pressure (PASP) can be estimated with Doppler echocardiography by measuring the maximum tricuspid regurgitation jet velocity (TRV).

PASP is estimated with the simplified *Bernoulli equation*: $PASP = (4 \times TRV^2) + RAP$ (124). The right atrial pressure (RAP) is an assumption of right atrial pressure, which is now commonly estimated by the size and respiratory variation of the flow in the inferior vena cava estimated with echocardiography. An inferior vena cava diameter ≤ 2.1 cm that collapses > 50% with inhalation suggests a normal RAP of 3 mm Hg (range 0-5 mm Hg) (125). Often when evaluating PASP in patients without right heart failure, a RAP is given a fixed value of 5 to 10 mmHg, but that obviously will overestimate the real PASP in most of the patients.

Earlier studies have suggested that PH is likely if the estimated PASP is > 50 mmHg and TRV > 3.4 m/s; on the other hand PH is unlikely if the estimated PASP is ≤ 36 mmHg and TRV ≤ 2.8 m/s (124, 126-128). The correlation between pulmonary pressures determined by echocardiography and by RHC is modest, with the risk of both a false negative but mostly false positive investigations (127, 129).

According to the 2009 European Society of Cardiology (ESC) and the European Respiratory Society (ERS) (124), pre-capillary pulmonary hypertension that is generally referred to as PH is defined as a mean pulmonary arterial pressure (mPAP) of 25 mm Hg or more at rest, coinciding with a pulmonary capillary wedge pressure (PCWP) within normal limits as 15 mm Hg or less. When physiological tricuspidal insufficiency is not apparent it is not possible to evaluate the TRV and thus not possible to evaluate PASP by echocardiography. Then additional echocardiographic features are necessary for determination, such as right ventricle and/or atrial enlargement, and/or other specific echocardiographic or clinical symptoms or findings suspect of PH.

Data from the national PAH registry in the United Kingdom with a population of about 60 million have estimated that the overall prevalence of PAH associated with connective tissue disease was 4.23 per million (130). The prevalence of PAH associated with SSc (PAH-SSc) was 2.93 per million and a total of 36 MCTD-PH patients were identified, giving the prevalence of MCTD-PH of approximately 0.6 per million.

Three of the four studies in MCTD were cohort follow-up studies from a single center and estimated the prevalence of PH at 23-24% (7-9). The fourth study was a multicenter screening study that estimated the prevalence of PH at 19% (18/94) solely based on echocardiography (20) where the PH was defined as PASP > 40 mmHg and importantly the definition of RAP was estimated either by the inferior vena cava diameter (125) or by fixed value.

**Table 3. The New York Heart Association functional classification. (114)**

| | |
|---|---|
| **Class I** | Without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain. |
| **Class II** | Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain. |
| **Class III** | Marked limitation of physical activity. Comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain. |
| **Class IV** | Inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased. |

**Table 4. Functional classification of pulmonary hypertension according to the WHO 1998* (115)**

| | |
|---|---|
| **Class I** | Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope. |
| **Class II** | Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope. |
| **Class III** | Patients with pulmonary hypertension resulting in slight limitation of physical activity. These patients are comfortable at rest, but ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope. |
| **Class IV** | Patients with pulmonary hypertension with inability to perform any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnea and/or fatigue may be present at rest, and discomfort is increased by any physical activity. |

*—Modified from the New York Heart Association classification of patients with cardiac disease.(114)

In 1998, the Japanese Ministry of Health and Welfare's MCTD Research Committee revealed that 5.0% of MCTD patients had PH, whereas PH was found in 0.90% and 2.64% in SLE and SSc, respectively. The report is in Japanese and not accessible and is not published in medical literature and it is practically impossible to conclude anything about these prevalence/frequency figures, although sporadically referred to by Japanese speaking authors (131).

There are obviously conflicting data on the prevalence and the pattern of PH, the most severe and threatening disease complication of MCTD. From previous reports (7-9), PH appears to evolve both early and late in the disease course and have a poor prognosis. Data from two national PAH databases have indicated that the one-year (130, 132) (89-88%) and three-year survival rates (130) (63%) of MCTD patients with isolated PAH were similar to those with isolated PAH associated with SSc. Several studies have shown that PH patients with ILD (PH-ILD), either with idiopathic lung disease (133, 134) or ILD associated with SSc (130, 132, 135), have significantly reduced survival compared to those with isolated PAH. Patients with PH-ILD tend to have lower oxygen saturation and frequently require oxygen supplementation, and PH diagnosis is usually made late, with the majority of the patients in the WHO functional class III-IV at the time of diagnosis (87%, 80% and 95%) (130, 136,

137). Worsening of oxygen saturation during follow-up is documented as a negative predictor in survival of SSc associated with PH-ILD (138).

There are no clinical trials or studies viewing the prognosis of subgroups of MCTD patients with PH. The outcome in clinical studies is currently mainly based on 6MWT and changes in dyspnea NYHA or WHO functional classes (Tables 3-4)(139).

Importantly, as observed in SSc, MCTD patients may well have both ILD and pulmonary vascular disease. In these patients it may be very difficult to establish whether the PH is an isolated vascular disease (PAH) independent of the ILD or whether it is caused by the ILD. Hence, it is currently uncertain to what extent PAH and PH-ILD represent two different phenotypes of PH in MCTD.

### 1.5.11 Pulmonary manifestations of MCTD

Pulmonary involvement is one of the five 'major criteria' of criteria by Sharp (6), defined as either reduced CO diffusion capacity ($D_LCO$) and/or proliferative vascular lesions in the lungs and/or pulmonary arterial hypertension. Pleuritis is among the eleven 'minor criteria'. Pleuritis (and/or pericarditis) is one of five 'SLE-like findings' in criteria by Kasukawa (5). In addition, in the Kasukawa criteria, lung involvement is included by either restrictive changes of the lung as a vital capacity (VC) less than 80% of predictive value and/or reduced predictive $D_LCO$ less than 70% of predicted value. However, lung involvement is not included in neither the Alarcón-Segovia (4) nor the criteria of Kahn (13).

In the study of Nimelstein and coworkers (28) analyzing the original 25 patients reported by Sharp et al. (3), serositis defined as pleuritis and/or pericarditis was reported in a total of 36%. In the study by Sullivan and coworkers investigating pulmonary involvement of MCTD, pulmonary disease was found in a total of 85% (29/34), including pleuritis in 35% (12/36). Resting hypoxemia was found in 21% (7/33) and reduced (defined as less than 75% of predicted value) single breath gas diffusion capacity was found in 72% of the tested patients (23/32) and reduced vital capacity in 41% (13/32). Data on high resolution computed tomography (HRCT) were not available but abnormalities were reported in 10/33 of initial chest x-rays interpreted as interstitial lung disease. Multiple transbronchial biopsies showed mild interstitial fibrosis in three patients 9% (3/34) and minimal histological changes in 12% (4/34). An open lung biopsy in one patient showed marked muscular hypertrophy of the small pulmonary arterioles. Three of the four patients that died showed marked intimal proliferation

and medial hypertrophy of pulmonary arteries and arterioles with consequent narrowing of the vascular lumens. In a prospective study by Lundberg (17) of 17 MCTD patients, 29% (5/17) had impaired pulmonary function defined as reduced vital and/or decreased CO diffusion capacity less than 75% of predicted values or signs of pulmonary fibrosis on chest x-ray. A total of 18% (3/17) had serositis. Pleuritis and/or pericarditis were reported in 19% on initial presentation and cumulative in 43% in the long-term hospital cohort study of Burdt et al. (8). Pulmonary dysfunction was not reported as an initial presentation in any patient but cumulative findings showed that 66% had pulmonary dysfunction in the course of the disease.

There are case reports of diaphragm dysfunction in MCTD as would be expected in patients with 'shrinking lung', commonly associated with SLE (140). The pathogenesis is considered a respiratory muscle involvement (141) or phrenic nerve involvement and there are cases of documented phrenic nerve neuropathy as phrenic nerve paralysis (142).

In conclusion, lung manifestations seem to be commonly associated with MCTD but most of the studies that have been carried out are relatively small. ILD seems to be most common lung manifestation in MCTD, and this will be reviewed in detail later.

**Interstitial lung disease**

There is an increasing awareness of ILD as an important cause of morbidity and mortality in systemic connective tissue diseases. In MCTD, focus on ILD appeared soon after MCTD was first introduced by Sharp in 1972. Bennett et al. (19) found that pulmonary disease was a common feature of MCTD. Pleurisy occurred in 30% and 90% showed a significant reduction in carbon monoxide transfer ($D_LCO$) that is associated with pulmonary involvement and the four patients that died in this study, died of pulmonary complications. Sullivan et al. carried out, nearly three decades ago, a prospective evaluation of pulmonary involvement in 34 patients with MCTD (9). A total of 85% of the patients had evidence of pulmonary involvement, two thirds (72%) had reduced $D_LCO$ (defined as less than 75% of predicted value) and 21% were hypoxemic, but only 30% had an abnormal conventional chest x-ray with a pattern of small irregular opacities predominantly involving the lower and middle lung fields. The results of HRCT of the lungs, were not reported. Burdt et al. (8) followed a cohort of 47 patients with MCTD for a mean of 15 years (3 to 29 years), of whom 66% cumulatively had an abnormal $D_LCO$.

Currently there are only five published studies utilizing HRCT of the lungs (Table 5, page 24) in the assessment of MCTD (24-27, 143). Three of the studies were retrospective (24-26) and three studies presented the results of pulmonary function testing (26, 27, 143). Four studies assessed the pattern of the HRCT changes (24, 25, 27, 143), but none included histological analysis of the lung, functional tests such as the standardized 6 min walking distance test (6MWT), functional classification by the NYHA or WHO functional classification, echocardiography or right heart catheterization.

Classification of ILD by high resolution computed tomography, by 2001, has been listed in the International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias, by the American Thoracic Society (ATS) and by the European Respiratory Society (ERS) (144). The ATS/ERS classification is based on histological changes and not assigned for use in CTDs such as MCTD. The earlier notion that a lung biopsy is a ‘gold standard‘ in the diagnosis of fibrotic lung disease is, however, not as clear as previously thought (145, 146). The term *‘honeycombing’*, is poorly defined (147). The terms used to categorize the idiopathic interstitial pneumonias (IIPs) were all derived from more than three decade old publications, terms like: *idiopathic pulmonary fibrosis* (IPF); *usual interstitial pneumonia* (UIP); *nonspecific interstitial pneumonia* (NSIP); *desquamative interstitial pneumonia* (DIP); *cryptogenic organizing pneumonia* (COP); *acute interstitial pneumonia* (AIP); *respiratory bronchiololitis-associated interstitial lung disease* (RB-ILD) and l*ymphoid interstitial pneumonia* (LIP). In addition the terms diverted attention from the pathogenesis and obscure the continuity between entities. For example UIP represents a histologic pattern that is entirely different from that originally described and DIP is no longer considered the result of desquamating type II pneumocytes (147, 148). When using HRCT/CT for analyzing lung involvement, the Fleischner Society glossary of terms for thoracic imaging (149) is recommended for classification of the findings.

**Table 5. Overview of studies on interstitial lung diseases using HRCT.**

| Study | Year | Country | No. pts. | F:M ratio | MCTD Criteria | Follow-up [years] | |
|---|---|---|---|---|---|---|---|
| Kozuka T et al.(24) | 2001 | Japan | 41 | 40 | Kasukawa | N/A | Retrospective HRCT analysis. 100% ground glass attention, 98% micronodules, 44% traction bronchiectasis, 37% honeycombing. |
| Saito Y et al. (25) | 2002 | Japan | 35 | 4.0 | Kasukawa | N/A | Retrospective HRCT analysis. 38 (38/73) normal or absent HRCT and 35 (35/73) abnormal HRCT. 100% septal thickening; 94% subpleural micronodules; 51% honeycombing and 11% ground glass opacity. |
| Bodolay E. et al. (26) | 2005 | Hungary | 144 | 12.1 | Alarcón-Segovia | 0.5 | 67% (96/144) had HRCT changes but after 6-8 week of methylprednisolone and/or combination with cyclophosphamide. Normalization of HRCT in 67/96. Total lung changes after 6 months in total 20%; ground class opacity with fibrosis in 10% (15/144); lung fibrosis in 9% (13/144) and honeycombing in 0.7% (1/144). |
| Aaløkken TM et al. (143) | 2009 | Norway | 24 jMCTD | 3.8 | Kasukawa | N/A | Screening of juvenile onset MCTD in median age of 23. Discrete ILD was identified in 6 pts (25%). With very mild disease in which 5% or less of the parenchyma was affected. |
| Fagundes MN et al. (27) | 2009 | Brazil | 50 | 49 | Kasukawa | N/A | Screening w/HRCT and PFT, esophageal manometry and pH monitoring. Total 78% had ILD, 72% ground glass opacities, 48% subpleural linear opacities, 24% traction bronchiectasis, 24% honeycombing, The presence of ILD was associated with esophageal involvement. |

PFT = pulmonary function tests, HRCT = high resolution computed tomography, ILD = interstitial lung disease

## 1.6 Treatment and outcome

### 1.6.1 Treatment

The initial assumption by Sharp and coworkers (3) that MCTD was responsive to corticosteroid therapy and yielded a favorable outcome has regrettably, not stood the test of time.

In spite of four decades since the disease concept of MCTD first was presented, no randomized controlled trials on therapies for MCTD have been performed. At present, the general remitting treatments is available. The therapy is commonly individualized according to the clinical manifestations, and is based on knowledge and experience of treating other related diseases and disease manifestations.

Symptomatic treatment of secondary RP, the most common symptom of the disease, consists of general advice (keep warm, avoid injury, smoking and caffeine) (150). Treatment with per oral calcium channel blockers that block voltage-gated calcium channels (VGCCs) in cardiac muscle and blood vessels is documented. Vasodilation decreases total peripheral resistance and increases peripheral blood flow with effects on RP (151). Nifedipine/Adalat® is commonly used but other dihydropyridine calcium channel blockers can be used, such as amlodipine/Norvasc®; felodipine/Plendil® or isradipine/Lomir® and others. Use of Ketanserine/Sufrexal®, a peroral antihypertensive drug, has been documented. This drug has highly complex pharmacological effects, mainly through moderate selectivity for 5-$HT_{2A}$ receptors over 5-$HT_{2C}$ receptors, in addition to a very high affinity for histamine H1 receptors and high affinity for alpha-1 adrenergic receptors (152-155). Ketanserine has only a modest clinical effect on Raynaud's phenomenon (153). Administration of intravenous prostaglandin, most often ilioprost/Iliomedin® has documented effects (150). Some case reports document a positive effect from topical nitroglycerin on RP, but for all other interventions, the current evidence is limited, conflicting or nonexistent (67, 150). There are currently no guidelines regarding treatment in MCTD but most physicians would prefer starting treatment with calcium channel blockers, such as nifedipine/Adalat®.

The treatment of digital ischemic/ulcers is difficult. There is documentation for the use of oral sildenafil/Viagra® or Revatio® (156, 157), prostaglandin infusions (158) and digital

sympathectomy (159, 160) in the acute phase. Oral use of the endothelin 1 receptor inhibitor, bosentan/Tracleer®, has a documented effect in reducing the risk of new digital ulcers in SSc (161-163), but none of these drug or other treatment options have documented efficacy in treating MCTD. Symptomatic NSAID treatment has commonly been used for arthralgia and myalgia and treating pain associated with pericarditis and pleuritis. Treatment of esophageal reflux and/or dysphagia consists of protein pump inhibitors, H2 antagonists and antacids.

Antimalarials, commonly per-oral hydroxychloroquine/Plaquenil® (HCQ), are by many considered a basic treatment for MCTD as for SLE, pSS and UCTD, but there are no trials to support this. For the more severe form of the disease, such as pleuritis, pericarditis, arthritis and/or severe interstitial lung disease, steroids, either orally or intravenously, are still one of the cornerstones of therapy, mostly in combinations with other treatments. Use of oral or intramuscular methotrexate or oral cyclosporine for arthritis and active myositis is documented for related diseases. Oral azathioprine (AZA) and mycophenolate mofetile (MMF) have been used for hemolytic anemia and autoimmune thrombocytopenia and interstitial lung disease. More serious manifestations of interstitial lung disease have been treated with intravenous cyclophosphamide, but there are no controlled trials for MCTD supporting this.

There are no specific guidelines in treatment of pulmonary hypertension associated with MCTD and the treatment options are mostly from treatment of PAH associated with SSc and/or treatment of idiopathic PAH. Treatment with calcium inhibitors, in the very few patients that respond to it, is the preferred treatment for pulmonary arterial hypertension (PAH). The patients are also commonly treated with anticoagulation, most often warfarin, to reduce the risk of intrapulmonary thrombosis and thromboembolism. The more specific treatment of pulmonary hypertension consists of endothelin receptor antagonists (bosentan, ambrisentan), phosphodiesterase 5 inhibitors (sildenafil, tadalafil or vardenafil) and prostanoids (epoprostenol, treprostinil, inhaled ilioprost). In treatment resistant cases a combination of two and even three of these treatment options is used. The most preferred therapeutic option is an endothelin receptor antagonist followed by phosphodiesterase 5 inhibitors.

There is little experience with biological treatment of MCTD. There are a few reports of both adult (164-167) and juvenile (168) MCTD cases treated with B cell depletion therapy with rituximab. There are several case reports of MCTD patients having several adverse effects

when being treated with tumor necrosis factor alpha (TNF alpha) inhibitors (169-171). There is one documented case of MCTD with therapy resistant pulmonary hypertension treated with tocilizumab, (172) a humanized monoclonal antibody to human IL-6 receptor.

### 1.6.2 Outcome

There are no uniform standardized outcome measures or damage measures for MCTD.
It is still debated whether MCTD represents a distinct disease entity or is an early form of other related connective tissue diseases (CTDs), such as SLE, SSc, DM/PM. Results from a few, small and heterogeneous cohort studies are conflicting, partly due to differences in disease definitions. These studies fall into two main categories: studies following patients having anti-RNP antibodies (17, 28, 41, 42) and studies following patients having both anti-RNP antibodies and a clinical diagnosis of MCTD (8, 17, 40, 41). The conclusions from these studies have been mixed, some indicating that the disease evolves into other known CTDs in the majority or even in all patients (28, 42), whereas others find that MCTD in most patients does not evolve into other CTDs (8, 17, 40).

In some of the studies following anti-RNP antibody positive patients (17, 28, 41, 42), no specific criteria for MCTD were used (28, 41) and in one study (42) 22% of the patients did not even have the diagnosis of MCTD and 10% of the patients classified as MCTD possessed anti-dsDNA antibodies (42). The inclusion methods in studies following patients with MCTD also differed. The patients either fulfilled specific MCTD criteria (4-6, 13) at inclusion (8, 17) or were retrospectively defined as MCTD by an expert panel (40).

Lundberg and Hedfors presented two decades ago a follow-up study for a mean of 122 month (range 33-318 months) of 32 patients with both low titers (9 patients) and high titers (23 patients) of anti-RNP antibodies (17). By analyzing the patients with high anti-RNP antibody titers at the inclusion, three (13%), and at the end of the study 17 (74%), fulfilled the proposed criteria of Alarcón-Segovia (4). None of these patients developed SSc, while two (2/17) had MCTD/SLE overlap and five patients with low titer anti-RNP developed SLE. In the long-term study by Burdt (8) 47 MCTD patients fulfilled the Kasukawa criteria (5); the follow-up was carried out for a mean of 15 years (range 3-29 years). This implies, contrary to earlier short-term studies, that the MCTD diagnosis was stable over time and that MCTD rarely evolved into SLE or SSc. Ten patients (21%) later fulfilled the preliminary classification criteria for SSc (173), whereas five (11%) still fulfilled the criteria of MCTD with serologic,

immunologic and genetic findings that were regarded as typical of MCTD rather than SSc. Five out of the six (13%) patients that fulfilled the revised SLE criteria (174) still met the criteria for MCTD. Thus in the long-term follow-up of 47 patients, the symptoms in five patients (11%) evolved to SSc and in one patient (2%) to SLE (8).

**Mortality**

Little is known about the mortality of patients with MCTD (29). Current knowledge is based on a handful of single-center follow-up studies with small cohorts consisting of 15-47 patients, with a mean observation time between 5-17 years. There are altogether eight studies (8, 9, 19, 28, 65, 175-177) presenting a total of 217 MCTD patients of whom 48 had jMCTD. The study covered a total of 43 deaths. Four studies (8, 9, 28, 176) had a time span longer than a mean of 10 years (11-17 years) of follow-up consisted of a total of 126 patients and reported 28 deaths that cumulatively gave a crude mortality rate of 22%, varying between 12 and 36% (8, 9, 28, 176). The causes of death in MCTD patients in these studies were mainly related to ILD and/or PH in addition to infections (8) and malignancies, but unlike SLE, not to cardiovascular disease (178, 179).

It is difficult to draw meaningful conclusions from these studies because of the heterogeneity of the patients, different follow-up times and the fact that there were no comparisons with matched controls from the general population. Clearly, further studies on mortality and other outcome measurements and phenotype stability in MCTD are warranted.

# 2 Aims of the study

## 2.1 General aim

The general aim of this nationwide, cross-sectional multicenter study was to identify all accessible living MCTD patients in Norway in order to describe epidemiological and clinical features of the disease and establish, as completely as possible, a nationwide Norwegian cohort of MCTD patients.

## 2.2 Specific aims

- To investigate the basic epidemiology, such as prevalence, incidence, sex ratio, age at both onset and diagnosis, and geographic distribution of the patients (paper I).
- To evaluate the most common clinical symptoms and findings in the course of the disease (paper I).
- To investigate the prevalence and pattern of interstitial lung disease in MCTD by screening the cohort with high resolution computed tomography (HRCT) scanning of the lungs, pulmonary function tests and functional testing (paper II).
- To evaluate the prevalence and morbidity of pulmonary hypertension (PH) by screening the cohort echocardiography and in selected cases perform a right heart catheterization (paper III).
- To explore and analyze possible differences between the three most used MCTD disease criteria (the modified Sharps criteria (9), the criteria by Alarcón-Segovia and Villareal (10) and Kasukawa et al. (13)) on the epidemiology of the disease (paper I), on pulmonary affection (paper II) and pulmonary hypertension (paper III).

# 3 Patients and methods

## 3.1 Patients

**Inclusion of patients**

Sixteen Norwegian public hospitals with Departments of Rheumatology agreed to participate in this nationwide, multicenter cross-sectional study. The inclusion of all available patients with MCTD fulfilling the inclusion criteria in Norway started the 15th of March 2005 and lasted to the 31st of December 2008.

The initial step of the study was case finding. The Norwegian Systemic Connective Tissue and Vasculitis Registry (NOSVAR) was used to find MCTD patients. Additionally, the hospital records were reviewed to identify patients. The tenth revised version of the World Health Organization (WHO) International Classification of Diseases (ICD10) coding system (http://www.who.int/classifications/icd/en/) has been in use in the Norwegian Hospitals since 1999. The ICD10 was the first version of the coding system in which the diagnosis MCTD had a separate code (M35.1).

The inclusion of patients was based on the five following criteria: age 18 years or older; a clinical diagnosis of MCTD verified by a rheumatologist; exclusion of other connective tissue diseases; documented high titer serum anti-ribonucleoprotein antibody (anti-RNP) test and fulfillment of at least one of three most commonly used criteria sets for MCTD – the modified Sharps criteria set (6), the criteria set of Alarcón-Segovia and Villareal (4) or the criteria of Kasukawa and co-workers (5).

Patients who satisfied all five inclusion criteria were given oral and written information about the study and asked to participate. The patients who provided an informed, written consent were then enrolled in the study.

All included patients accepted participation and there were no dropouts.

## 3.2 Data collection and analysis

The clinical assessment of each patient was performed according to a pre-defined clinical form by one of the investigators at inclusion. The assessment included: standardized questions

about symptoms, clinical examination and predefined blood tests. The presence of pleuritis, pericarditis and arthritis was based on clinical symptoms and findings, whereas the presence of myositis was defined by increased serum creatine kinase (CK) levels and signs and/or symptoms of proximal muscle weakness at time of the inclusion and/or in the course of the disease. The myositis was, in some cases, confirmed by muscle biopsy and/or electromyography. Additional investigations included echocardiography, lung imaging by HRCT, PFTs, and physical capacity was evaluated by the 6 minute walking test (116) and New York Heart Association (NYHA) functional classification (114) and serum samples for N-terminal pro-Brain natriuretic peptide (NT-proBNP).

**Laboratory tests**

Primary serum ANA and anti-RNP analyses of the patients were performed locally. To avoid potential false positive anti-RNP tests, only patients with high titer antibodies (as defined by their local laboratory) were included.

**Analyses of the HRCT lung scans**

Thin-section CT images were obtained in the supine position during breath-holding and deep inspiration. Supplementary expiratory and prone scans were obtained when indicated. Several different CT scanners at 10 hospitals were used. All the available HRCT images were reviewed in consensus and in random order, blinded to patient history and lung function, by two experienced chest radiologists.

The presence, extent, and distribution of ground glass attenuations, airspace consolidation, reticular patterns, and interlobular septal thickening were evaluated according to the CT criteria of ILD recommended by the Nomenclature Committee of the Fleischner Society (149). If ground-glass attenuations were superimposed on a reticular pattern, the abnormality was recorded as being reticular. The presence of associated findings, including traction bronchiectasies, nodules, pleural irregularities, cysts, emphysema, and air trapping, was also assessed. Since ‘honeycombing’ is a poorly defined parameter (147), it was not assessed.

The reticular patterns (i.e. the coarseness of the lung fibrosis) were graded as follows: 1, fine intralobular pattern without evident cysts; 2, a pattern with predominant small cysts involving air spaces smaller than or equal to 4 mm in diameter; and 3, a pattern with larger cysts (macrocysts) involving air spaces larger than 4 mm. When a reticular pattern grade 1 was superimposed on a grade 2 pattern, the abnormality was recorded as a reticular pattern grade

2. If different types of reticular patterns were present in different lung zones of an individual patient, both pattern types were recorded.

The distribution of disease was reviewed in four lung zones: (a) above the aortic arch, (b) between the aortic arch and the level of the carina, (c) between the level of the carina and the level of the inferior pulmonary veins, and (d) below the inferior pulmonary veins.

The extent of ground-glass attenuations and reticular patterns in each zone was assigned an area score based on the percentage of the lung parenchyma involved; 0, no involvement; 1, 1-4 % involved; 2, 5-14 %; 3, 15-29%; 4, 30-49%, and 5, more than 50% involved.

A total reticular pattern score was calculated by adding up the individual area scores (0-5) of the reticular pattern changes in each of the four lung zones. A total reticular score of 0 was defined as no fibrosis. A total reticular score of 1-2 was defined as minor fibrosis, 3-4 as moderate fibrosis and 5 or higher as severe fibrosis.

**Pulmonary function tests**

The PFT of each patient was performed within 6 weeks before or after the HRCT and included the forced vital capacity (FVC) and the forced expiratory volume in 1 second (FEV1). CO diffusing capacity ($D_LCO$) and the CO diffusing capacity divided by the alveolar volume ($D_LCO/VA$) were performed according to published guidelines (180-182). The PFT values were expressed in absolute terms and as % of the predicted value. Predicted values were derived from reference equations, separated by gender with age and height as predictor variables.

**Screening for pulmonary hypertension**

Echocardiographic examination was performed by a cardiologist at the patient's local hospital. The pulmonary artery systolic pressure (PASP) was estimated by Doppler echocardiography by measuring the maximum tricuspid regurgitation jet velocity (TRV). Assumption of right atrial pressure (RAP) can be estimated by the size and respiratory variation of the flow in the inferior vena cava (125) or using a fixed value of 5 to 10 mmHg in patients without right heart failure. PASP was estimated with the simplified Bernoulli equation: $PASP = (4 \times TRV^2) + RAP$ (124). Earlier studies have suggested that PH is likely if PASP > 50 mmHg and TRV > 3.4 m/s. PH is unlikely if PASP ≤ 36 mmHg and TRV ≤ 2.8 m/s (124, 126-128). Based on this fact, we decided to use PASP > 40 mmHg as a cut-off value for performing RHC, when PASP could be calculated. Patients with lower PASP who had additional echocardiographic features suggestive of PH, and/or other clinical symptoms

or findings suspected of having PH, were also referred for right sided heart catheterization (RHC) in accordance with the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) (124).

All MCTD patients suspected of PH were referred to RHC at Department of Cardiology in Oslo University Hospital Rikshospitalet, a center for diagnosis and treatment of patients with PH in Norway.

PAH and PH was defined according to the 2009 Guidelines for the diagnosis and treatment of PH (124) by the European Society of Cardiology (ESC) and the European Respiratory Society RS) and also endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Precapillary PH was defined as a mean pulmonary arterial pressure (mPAP) $\geq$ 25 mmHg at rest with a normal pulmonary capillary wedge pressure (PCWP) ($\leq$ 15 mmHg). The classification was in coherence with the updated clinical classification of pulmonary hypertension from the fourth World Symposium on PH held in Dana Point California in 2008 (117), where patients with connective tissue diseases and PH were classified into two groups: those without interstitial lung disease, as pulmonary arterial hypertension (PAH) (Dana Point Group1.4.1), and those with ILD, as pulmonary hypertension (PH-ILD) (Dana Point Group 3.2).

**Data analysis and management**

Updated geographic population data for Norway were gathered from the Norwegian Statistical Institute (Statistisk sentralbyrå, http://www.ssb.no). For incidence, the year at diagnosis was recorded retrospectively. The incidence rate was calculated as the number of patients per million inhabitants per year. As only patients 18 years and older were included, only the incidence of adult onset MCTD could be estimated.

Completed data forms were sent to the primary investigator at the Unit of Rheumatology, Oslo University Hospital Rikshospitalet, for central registration and analysis. Cardiff Teleform 10.1 scanning software (http://www.intelliscan.com/TeleForm1.htm) was used to import data to the Microsoft Office Access database software 2000/2007.

## Ethical permissions

The study was accepted by the Regional Committee for Research Ethics (REK) in the Southern and Eastern Norway Regional Health Authority and the Norwegian Social Science Data Services (NSD).

**Statistical analysis**

Differences between groups were tested by the independent sample t test, the Mann-Whitney U test, Mantel-Haenszel $\chi^2$ test or Fisher's exact test, as appropriate. All tests were two tailed, with a 95% significance level. Statistical analyses were performed using SPSS/PASW version 19 (http://www.spss.com/) and OpenEpi version 2.3.1 (http://www.openepi.com/OE2.3).

# 4 Summary of results

## 4.1 Paper I

A total of 147 adult Caucasian MCTD patients fulfilled the inclusion criteria. It was possible to chart the year of diagnosis in 99% of the patients. The time span from the clinical diagnosis of MCTD to study inclusion ranged from less than 1 year to 38 years. The mean follow-up time from diagnosis to inclusion was 12.8 years (95% CI 11.4 - 14.2 years). The clinical manifestations most frequently reported were Raynaud's phenomenon (present in 99% of the patients) and 'puffy hands' (in 93%). Other common manifestations included arthritis (79%) and symptoms of esophageal dysmotility (50%).

The female to male ratio was 3.3, without a statistically significant difference between the 132 adult onset and the 15 (10%) juvenile onset MCTD (jMCTD) patients, defined as those diagnosed before 18 years of age. The mean age of diagnosis of the jMCTD was 13.0 years (95% CI 11.6 – 14.4 years) and the youngest patients were nine years old at diagnosis. The remaining 132 patients with adult onset MCTD had a mean age at diagnosis of 37.9 years (95% CI 35.3 - 40.4 years).

At the end of 2008, the point prevalence of living adult MCTD patients in Norway was 3.8 (95% CI 3.2-4.4) per 100,000 adults. The incidence of adult onset MCTD in Norway during the period 1996 to 2005 was 2.1 (95% CI 1.7-2.5) per million per year.

The point prevalence showed no statistical difference between the three criteria sets. Using the modified Sharps criteria (6) the point prevalence was 3.7 (95% CI 3.1 – 4.3) per 100,000, whereas using the criteria sets of Alarcón-Segovia (4) 3.5 (95% CI 2.9 - 4.1) and that of Kasukawa (5) 3.3 (95% CI 2.7 – 3.9) per 100,000.

We observed a distinct geographical variation in the prevalence of MCTD in Norway. The estimated point prevalence of adult MCTD in the Northern Norway Regional Health Authority (with a population of 0.46 million) was 1.7 (95% CI 0.3 – 3.0) / 100,000, while the estimated prevalence in the Southern and Eastern Norway Regional Health Authority (with a population of 2.67 million) was 4.5 (95% CI 3.6 -5.4) / 100,000.

The main conclusions were that MCTD has a female predominance, that the incidence and prevalence is low, and lower than that of other related connective tissue diseases such as PM,

DM, SSc and SLE. The prevalence estimates were similar across the three criteria sets (4-6) of MCTD.

## 4.2 Paper II

Previous, small, single-center reports have indicated that ILD is one of the most serious disease complications in MCTD. Here, a cross-sectional, interdisciplinary approach was used to identify and classify the type, distribution and extent of the HRCT changes and finally evaluate the impact of pulmonary disease in a nation-wide unselected MCTD cohort.

All the 147 MCTD patients in the cohort were referred to HRCT as part of the screening at inclusion, but only those that had HRCT files accessible for review were included in the study (86% or totally 126 patients). In spite of the fact that the majority of the excluded 21 patients had had an HRCT, these were not accessible for review. An internal analysis showed that there were no statistical differences between the 21 excluded patients and the 126 included patients regarding mean age, sex ratio, mean disease duration, overall disease manifestations, smoking status and mortality.

Patients were examined by pulmonary function tests (PFT); the six minute walking distance test (6MWT) (116); and by the New York Heart Association (NYHA) functional classification of dyspnea (114). The extent and type of HRCT lung abnormalities were scored according to the CT criteria of ILD recommended by the Fleischner Society (149).

The results showed that 52% of the patients had abnormal HRCT findings, most commonly reticular patterns consistent with lung fibrosis (35%). Lung fibrosis was quantified as minor in 7%, moderate in 9% and severe in 19% of the patients. Fibrosis was uniformly concentrated in the lower parts of the lungs and was not associated with smoking. Patients with severe lung fibrosis had lower PFT values, shorter 6MWT and a higher mean NYHA functional class. After a mean 4.2 years of follow-up, overall mortality was 7.9%. Mortality in patients with a normal HRCT was 3.3%, as compared to 20.8% in patients with severe lung fibrosis ($p < 0.01$).

The main conclusions were that severe lung fibrosis is common in MCTD, has an impact on pulmonary function and overall physical capacity, and is associated with increased mortality.

## 4.3 Paper III

Patients with MCTD are reported to develop pulmonary hypertension (PH), but there are limited data on the prevalence of this serious complication. Here, the aim was to assess the prevalence of PH in the nationwide, unselected MCTD cohort of 147 patients. The patients were screened at study inclusion by echocardiography and by high resolution computed tomography (HRCT) and PFT to evaluate ILD, 6MWT (116) and by the NYHA functional classification of dyspnea (114) and N-Terminal Pro Brain Natriuretic Peptide (NT-proBNP) in the blood . An estimated systolic arterial pressure above 40 mmHg was set as the predefined cut-off for referral to right side heart catheterization (RHC) using the 2009 criteria of the European Society of Cardiology and the European Respiratory Society (22). After the inclusion, the patients were followed clinically for a mean period of 5.6 years.

At inclusion, 2.0% (3/147) had established PH. Echocardiographic screening at inclusion identified possible PH in six additional patients, of whom five were examined by RHC, and none of them had PAH. Two additional PH patients were identified during follow-up, giving a total PH frequency in the cohort of 3.4% (5/147). Two had isolated pulmonary arterial hypertension (PAH) and three PH associated with interstitial lung disease (PH-ILD). None of them had PH owing to left heart disease or chronic thromboembolism. Three PH patients died during follow-up. Nine other patients in the cohort also died, but none of them had echocardiographic signs of PH prior to death.

The usefulness of the biomarker NT-pro Brain Natriuretic Peptide (NT-proBNP) measurement in blood in PH in MCTD was also confirmed, as all five patients with PH had elevated NT-proBNP at the time of inclusion or at the time of the PH diagnosis. The main conclusions were that the prevalence of PH in MCTD was lower than expected from previous reviewed studies and support the previous notion that PH is a serious complication with high mortality.

# 5 General discussion

During the past decade, the research activity on MCTD has been low compared to that of other related connective tissue diseases. To put this into context, there are currently (1st of June 2012) 2,220 references on PubMed® (http://www.ncbi.nlm.nih.gov) using MCTD and/or mixed connective tissue disease as keywords, compared with a total of 54,770 references on SLE and/or systemic lupus erythematosus and 23,640 references on systemic sclerosis and/or scleroderma. Regrettably there are few recent studies focusing on MCTD. The vast majority of the references on MCTD are case presentations and diverse review articles touching a wide spectrum of themes around CTDs where MCTD is mentioned.

***Prior to the current project:***

- The knowledge of MCTD has largely been based on several small, mostly two to three decade old cohort tertiary medical center studies (7-9, 15, 17, 19). Several of these studies did not use (7, 9, 19, 28, 41, 42, 65, 66) any of the four proposed criteria of the disease. Several studies mixed patients with the existence of anti-RNP antibodies with patients with an MCTD diagnosis and in some of the studies even included patients diagnosed and fulfilling criteria of other connective tissue diseases such as SLE, RA, DM/PM and/or SSc at the time of inclusion (41, 42, 52). These studies found, perhaps not surprisingly, that many of the included patients did not have MCTD at all.
- There were no epidemiological data on MCTD, neither on prevalence or incidence.
- The prevalence, severity and pattern of interstitial lung disease in MCTD were unknown.
- The prevalence of pulmonary hypertension (PH) with (PH-ILD) and without (PAH) lung disease was unknown, in spite of textbook knowledge (183) stating that pulmonary hypertension (PH) in MCTD is a major disease complication occurring in at least 23% (183).

## 5.1 Patients and methods

When planning the nationwide Norwegian MCTD study we analyzed different theoretical research approaches. One of the alternatives was to identify patients with high anti-RNP antibodies proven by auto-immune laboratories and further examine these for the disease

criteria of MCTD. The main disadvantage of this approach is that it is currently unclear if these data are available or accessible. Moreover, there are several laboratories using different analytical methods over a span of several years. Enormous resources would be needed to properly sort out patients with only positive anti-RNP test from MCTD patients. Another alternative research approach would be to perform a population based screening. That clearly would need huge resources. Rare diseases such as MCTD are generally not suitable for population based screening, and it would create problems both regarding non-responders and sorting out a huge number of false positive candidates. Because of the rarity and complexity of MCTD, we decided to perform a multicenter study with the cooperation of departments of rheumatology in Norway with the possibility of detecting geographical differences in the epidemiology and/or clinical features of MCTD.

Before the start of the study a consensus meeting was held with all participating doctors, reviewing the research plan and the research sheets. The aim was to adopt unanimous understanding of the definitions when filling the research sheet.

The current research project used a classical epidemiological clinical study approach, with exclusion and inclusion criteria and a predefined research/screening plan with a questions sheet with registration of current and retrospective clinical variables based on a combination of documented information by reviewing the patient clinical information, and reviewing with the patients their current and earlier symptoms, and screening patients. The retrospective aspect of the study was focused on the year of debut and the year of diagnosis for retrospectively estimating the incidence rate, and in addition to pinpoint the stability of the MCTD disease phenotype. This approach has its limits. The patient had to be diagnosed with MCTD and traceable in the diagnosis registry, and the variables that were tested for have to be predefined.

The first part of the project was case finding. The Norwegian Systemic Connective Tissue and Vasculitides Registry (NOSVAR) was utilized to find MCTD patients, in addition to the main sources of finding patients, the patient registry systems in the hospitals. Patient contacts in the public health care system in Norway are coded with the diagnosis. The tenth version of the World Health Organization (WHO) International Classification of Diseases (ICD10) coding system has been in use in Norwegian hospitals since 1999. The ICD10 is the first version where MCTD had its separate code (M35.1). The case finding part of the study had an obvious weakness and detection was of course dependent on the fact that the patients had

received the diagnosis of MCTD prior to the study. The current study did not include patients not reviewed and diagnosed with MCTD, and how many these in fact are is currently only speculative. The diagnosis of MCTD is complicated and the diagnosis would supposedly and most frequently be carried out by rheumatologists or doctors with experience in rheumatology. In the health care system in Norway, most patients are diagnosed and controlled by either the in- or out-patient practice of the public hospital rheumatology care. The missed patients would presumably be those with minor disease activity followed by the primary health care practitioners and would not be getting rheumatology specialist care or those with disease manifestations mainly from one organ that would be followed primarily by other specialists, such as pulmonologists or cardiologists. In addition to the former two groups of patients those MCTD patients who were newly diagnosed during the prolonged period of inclusion in the study could have been missed in the study.

In the second part of the case finding step, the patients were individually reviewed for inclusion. Patients were included based on the five criteria as earlier reviewed in detail. We did not systematically file the patients who did not fulfill the entry requirements or those excluded. The reason for this was the multicenter nature of the study and when planning the study that was regarded as complicating the including process. The impression is that the most common reason was a false registration in the hospital own file system. There were few patients that had clinical diagnosis of MCTD that did not fulfill one of the three criteria, but those were not accounted for. The total number of those is currently unknown but the impression is that those were few.

When reviewing MCTD one of the inclusions criteria fulfillment of at least one of three most commonly used criteria sets for MCTD: the modified Sharp's criteria set (6), the criteria set of Alarcón-Segovia and Villareal (4) or those of Kasukawa and co-workers (5); The fourth MCTD criterion by Kahn (13) is very similar to that of Alarcón-Segovia. In addition all patients that fulfill Kahn's criteria also fulfill Alarcón-Segovia criteria (Table 1, pages 2-3) and it is meaningless to include both.

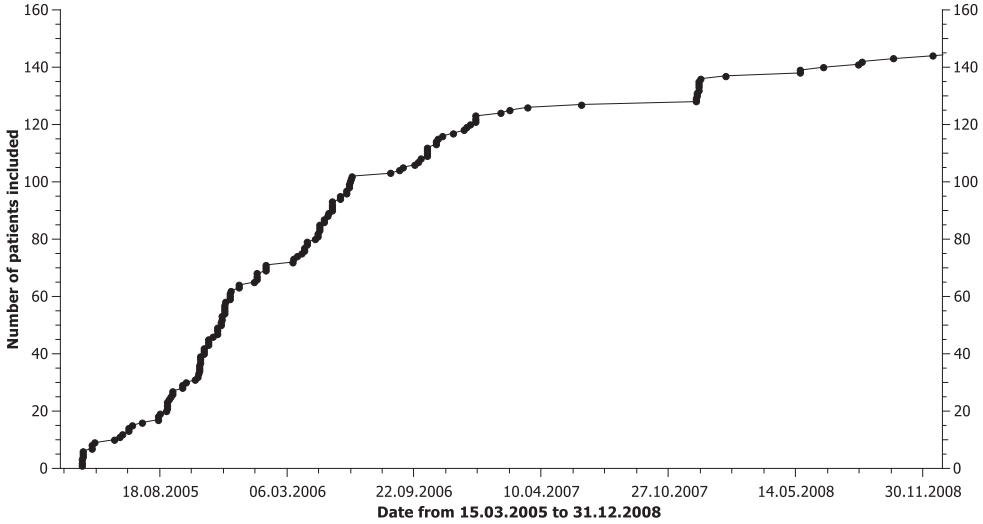
The other reason for not including patients was denial from the patients to participate was apparently extremely rare, but there are few reported patients that did not want to participate in part of the studies such as RHC or 6MWT.

The diagnostic terms that are used to classify patients with autoimmune diseases have changed throughout the years. It is challenging to classify complicated immunological processes that also change individually over time. The concept of MCTD has been debated through the years and it is possible that patients that could have been classified as MCTD are hidden behind other related CTDs diagnosis. As mentioned earlier, patients diagnosed with other CTDs diagnoses were excluded from the study. However, it is also possible that patient included in the study as MCTD could fulfill classification criteria of other related CTDs either at inclusion or over time.

Earlier studies on MCTD mainly used either no MCTD criteria, the criteria sets of Kasukawa (5) or those of Alarcón-Segovia (4). The criteria of Sharp (6) and those of Kahn (13) have rarely been used in clinical studies, the former because of the complexity and the latter as they are very similar to the Alarcón-Segovia criteria (4) One of the reasons for using three different disease criteria was that Kasukawa's (5) and Sharp's criteria (6), on the one hand, preferentially select a subset of patients with potentially more serious organ involvement such as lung involvement, pericarditis/pleuritis, PH, leucopenia and thrombocytopenia, whereas Alarcón-Segovia's (4) and the similar Kahn's criteria (13), simply encompass joint and muscle involvement in addition to Raynaud's phenomenon.

A systematic screening for interstitial lung disease and pulmonary hypertension was performed with a standardized 6 minute walking testing (6MWT) (116); to ensure that this was carried out in as nearly the same way as possible in all the centers, a film with detailed procedures was distributed to the participating centers. The patients underwent a complete pulmonary function testing (PFT), high resolution computed tomography (HRCT) and echocardiography at each registration center. Ideally, all these tests should have been done in the same laboratory, with the same CT scanner and the same respiratory laboratory and to perform standardized echocardiography investigations rather than those based on clinical screening. On the other hand, the selected cases suspected of PH were admitted to Oslo University Hospital, Department of Cardiology, for right heart catheterization. The inclusion in the study started in the middle of March 2005 and the initial plan was to finish including participants at the end of 2006, but the inclusion time was extended twice until the end of 2008. The reason for the extensions was the difficulties in collecting patients from different parts of the country as one of the main goals was to include all available patients from the whole country and thus have a complete, nationwide and large MCTD cohort (Figure 2).

**Figure 2: Cumulative inclusion of the 147 patients in the nationwide Norwegian MCTD study from the 15th of March, 2005, to the 31st of December, 2008.**




## 5.2 Results

**Epidemiology**

In the first article (paper I) (1), the results of both prevalence and incidence could possibly be underestimated. The hospital-based strategy chosen has some potential weaknesses. First, it could miss undiagnosed MCTD patients treated by other medical specialties. This is probably a minor problem in Norway, since the majority of patients with suspected connective tissue diseases are referred to one of the public departments of rheumatology. Second, the selected strategy would miss patients who died before inclusion and also those who developed SLE, inflammatory myositis or, systemic sclerosis before inclusion. Third, this strategy could miss MCTD patients who were lost to follow-up but that were mainly patients diagnosed prior to 1999/2000, i.e. prior to ICD10 coding in the hospitals. Finally, the inclusion in the study was extended by two years. The incidence figures of the disease that were retrospective could also underestimate the real incidence as only adult onset incidence of MCTD was estimated.

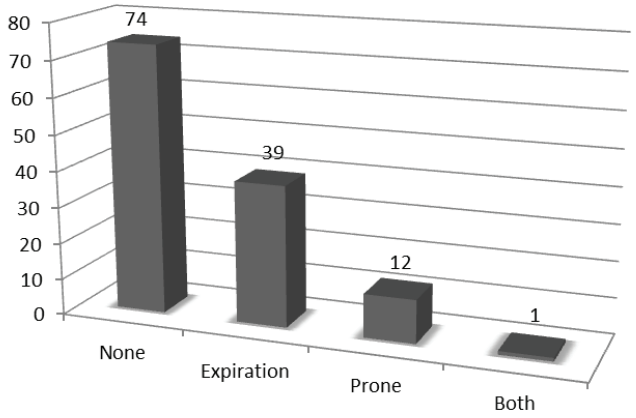
The main conclusion was that the MCTD was a very rare disease with lower estimated incidence and prevalence than SLE, SSc, pSS, PM and DM. The disease had a female

predominance with a female to male ratio of 3.3, a less pronounced female dominance than previously suggested (Table 2, page 8).

**Interstitial lung disease**

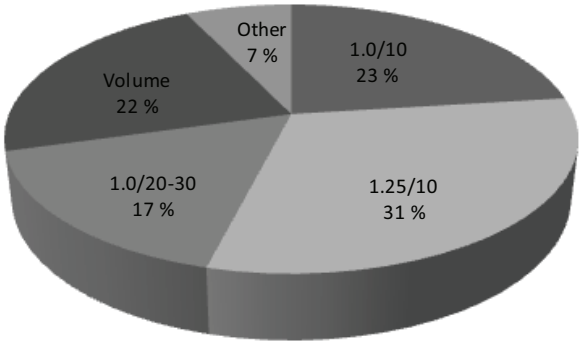
The difference in the disease criteria became apparent in the second paper (paper II) of this thesis which evaluated the prevalence and degree of ILD (2) as statistically fewer with severe lung fibrosis fulfilled the Alarcón-Segovia criteria (4) compared to Kasukawa's criteria (5). With regard to ILD (2), one of the weaknesses in our study was that the HRCT investigations and the PFT were done by different HRCT scanners by different methods (Figure 3 and Figure 4, page 44) and in different respiratory laboratories. This was, however, an inevitable consequence of the multicenter approach of the study. However, several factors minimalize these weaknesses. All the HRCT were done in the departments of radiology and the PFT at the hospitals respiratory laboratory by the departments of pulmonology mostly at university hospitals. In addition, the interpretation of the original HRCT files was centralized and performed individually by two experienced chest radiologists.

**Figure 3. Extra projections by high resolution computed tomography (HRCT) of the participants (n=126). (2)**




*None:* Standard projections taken during breath-holding and in deep inspiration in a supine position.
*Expiration: In* addition to standard projections there were HRCT projections during breath-holding in deep expiration.
*Prone:* In addition to standard projections there were HRCT projections of the patients lying in a prone position.
*Both*: In addition to standard projections there were HRCT projections taken during breath-holding in deep expiration in a supine position in addition to projections of the patient lying in a prone position.

**Figure 4. The HRCT method in the patients included in the MCTD – ILD project (n=126). (2)**




The various thickness of the HRCT slices and the method of HRCT processing (1) *1.0/20-30* [1.0 slices with 20-30 mm interval] (2) *1.0/10* [1.0 slices with 10 mm interval], (3) *1.25/10* [1.25 mm thick slices with 10 mm interval] (4) *Volume* (5) *Other* methods.

Of the 147 patients included in the study, 21 original HRCT files were missing and these patients were excluded from further examinations. There was no statistical difference between the 126 and the 21 patients whose HRCT files were not accessible (Table 6, page 45). That supports the claim, that the patients investigated and reviewed with HRCT, were representative for an unselected cohort of MCTD patients.

There is currently no consensus on how lung fibrosis should be defined by HRCT (184-186). The American Thoracic Society/European Respiratory Society (ATS/ERS) criteria for idiopathic interstitial pneumonias (144) were not applied. As described in section 3.2. and in *Paper II* (2), we used the recommended classification by the Committee of the Fleischner Society (149). There were several reasons for this choice. The ATS/ERS classification is mainly based on histology and ideas about pathogenesis, some even outdated (page 23). Connective tissue diseases are not included in the ATS/ERS classification and are referred to as collagen vascular disease. The Fleischner Society classification, on the other hand, focuses on CT based findings. As HRCT was the main outcome measurement from analysis of MCTD regarding ILD, the Fleischner Society glossary of terms was chosen for evaluating the HRCT classification (149).

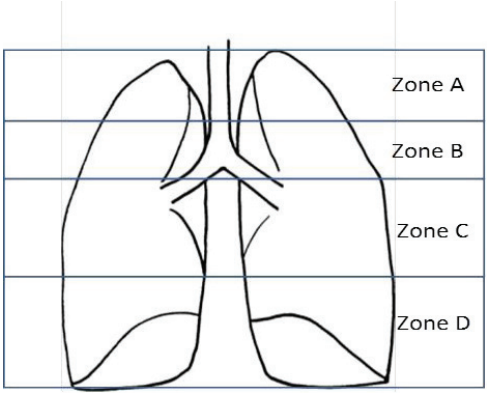
**Table 6. The total population of 147 patients, with 21 patients without available HRCT files and the 126 patients with accessible HRCT files.**

| | HRCT N/A Number pts | HRCT N/A Percent (%) | HRCT available Number pts | HRCT available Percent (%) | Total | |
|---|---|---|---|---|---|---|
| **Total number of pts.** | 21 | 14.3 % | 126 | 85.7 % | 147 | |
| **Male** | 2 | 9.5 % | 31 | 24.6 % | 33 | *NS* |
| **Female** | 19 | 90.5 % | 95 | 75.4 % | 114 | *NS* |
| **Juvenile MCTD** | 3 | 14.3 % | 14 | 11.1 % | 17 | *NS* |
| **Unknown smoking** | 1 | 4.8 % | 28 | 19.0 % | 29 | *NS* |
| **Non-smoker** | 11 | 52.4 % | 50 | 39.7 % | 61 | *NS* |
| **Earlier smoker** | 6 | 28.6 % | 24 | 19.0 % | 30 | *NS* |
| **Current smoker** | 3 | 14.3 % | 24 | 19.0 % | 27 | *NS* |
| **Weight [kg]** | 69.2 | [60.7 - 77.8] | 69.2 | [66.6 - 71.8] | | *NS* |
| **Height [cm]** | 165.5 | [162.7-168.3] | 169.3 | [167.9-170.9] | | *NS* |
| **Body Mass Index** | 25.1 | [22.6 - 27.7] | 24.0 | [23.3 - 24.7] | | *NS* |
| **Age at diagnosis[year]** | 35.6 | [22.7 - 43.4] | 34.8 | [32.0 - 37.7] | | *NS* |
| **Kasukawa criteria set** | 21 | 100.0 % | 106 | 84.1 % | 127 | *NS* |
| **Sharp's criteria set** | 20 | 95.2 % | 122 | 96.8 % | 142 | *NS* |
| **Alarcón-Segovia criteria set** | 18 | 85.7 % | 114 | 90.5 % | 132 | *NS* |
| **Mortality the 1st of January 2011** | 1 | 4.8 % | 10 | 7.9 % | 11 | *NS* |

*NS:* not significant by two tailed Fisher's exact test, with a 95% significance level, N/A= Not applicable, pts.= patients, HRCT= high resolution computed tomography

In the lung study the lungs were analyzed in four separate areas or zones having distinct anatomical boundaries (Figure 5).

**Figure 5. The four lung zones (A-D).**




**Above the aortic arch.**

**Between the aortic arch and the level of the carina.**

**Between the level of the carina and the level of the inferior pulmonary veins.**

**Below the inferior pulmonary veins**

Each of the four lung zones was quantified separately in 6 classes, based on percentage of lung parenchyma involved, by two experienced chest radiologists. Earlier studies had showed

that it was a good and validated method with excellent inter-observational agreement (146). The total changes in the 126 reviewed HRCT investigations by the Nomenclature Committee of the Fleischer Society (149) are presented in Table 7, but it is important to note that each patient could have more than one kind of HRCT changes.

**Table 7: Distribution of the HRCT abnormalities in lung zones A-D.**

| HRCT findings: | No. Pts. | % | Zone A[1] | Zone B[1] | Zone C[1] | Zone D[1] |
|---|---|---|---|---|---|---|
| **Normal HRCT** | **61** | **48 %** | | | | |
| **Abnormal HRCT** | **65** | **52%** | | | | |
| **Ground-glass attenuation** | **2** | **2 %** | 1 (1%) | 1 (1%) | 0 (0%) | 1 (1%) |
| **Reticular pattern type 1** | **27** | **21 %** | 10 (8%) | 14 (11%) | 16 (13%) | 20 (16%) |
| **Reticular pattern type 2** | **20** | **16 %** | 10 (8%) | 11 (9%) | 15 (12%) | 18 (14%) |
| **Reticular pattern type 3** | **7** | **6 %** | 4 (3%) | 3 (2%) | 6 (5%) | 6 (5%) |
| **Interlobular septal thickening** | **10** | **8 %** | 3 (2%) | 6 (5%) | 7 (6%) | 10 (8%) |
| **Nodules** | **7** | **6 %** | 6 (5%) | 7 (6%) | 5 (4%) | 5 (4%) |
| **Bronchiectasies** | **11** | **9 %** | | | | |
| **Air trapping** | **1** | **1 %** | 1 (1%) | 0 (0%) | 0 (0%) | 0 (0%) |
| **Emphysema** | **8** | **6 %** | 8 (6%) | 8 (6) | 5 (4%) | 5 (4%) |

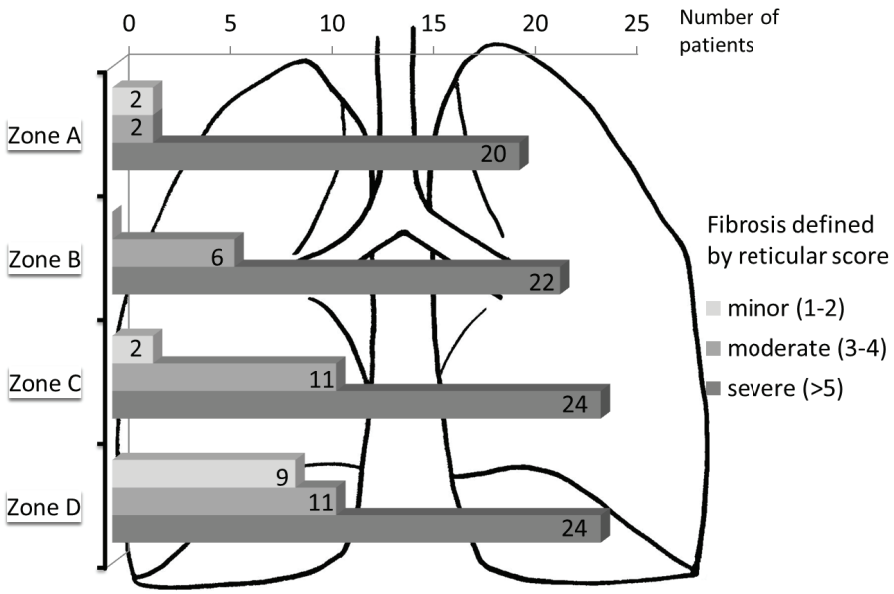
[1] Lung zone A: above the aortic arch. Zone B: between the aortic arch and the level of the carina. Zone C: between the level of the carina and the level of the inferior pulmonary veins. Zone D: below the inferior pulmonary veins.

In the radiological scoring system applied, the reticular changes were always defined as dominant, meaning that an area with ground-glass attenuations superimposed on reticular changes were recorded as an area with reticular changes. This is possible reason for the low frequency of ground-glass attenuations in our study compared to previous studies (24, 25, 187). Another, not mutually exclusive possibility, was the differences in disease activity at the time of sampling. Since ground-glass attenuations are mainly thought to reflect acute inflammatory processes in the lungs, they are more likely to occur in patients admitted to a hospital (due to infections or flares of increasing disease activity) whereas the other studies used for comparison were retrospective (24-27).

The reticular patterns identified in MCTD resembled the HRCT pattern seen in patients with nonspecific interstitial pneumonia (NSIP). The reticular pattern is considered to represent fibrosis and the coarseness of the fibrosis was graded into three classes: *reticular pattern type 1* with fine intralobular pattern without evident cysts; *reticular pattern type 2* with a pattern with cysts smaller or equal to 4 mm in diameter involving the air spaces; and finally *reticular pattern type 3* with cysts larger than 4 mm involving the air spaces. The lung zones were

individually graded in 6 classes from 0 to 5 for each of the three reticular patterns based on the percentage of the lung parenchyma involved (0, no involvement; 1, 1-4% involvement; 2, 5-14%; 3, 15-29%; 4, 30-49%, and 5 ≥ 50%). By adding the individual scores from each of these three reticular patterns for each of the four lung zones individually and then finally adding these together produced the total reticular score. Arbitrarily, those classified with a total reticular score of 1-2 were classified as having minor fibrosis, those with scores between 3 and 4 as having moderate fibrosis, and finally those with 5 or higher as having severe fibrosis (figure 6, page 47). It seems to be difficult to separate those differentiate between minor and moderate fibrosis in this study. Interestingly, even though the cut-off values for the total reticular score were arbitrary, it appeared that the patient subset which had a score above five (defined as severe lung fibrosis) had lower mean PFT values, shorter mean 6MWT and a higher mean NYHA functional class than the other subsets. This patient subset also had the highest mortality rate. The strong associations observed between the reticular score, the functional tests and mortality argue that the scoring system applied is valid but it has of course to be evaluated further (2); on the other hand, the distribution of the total reticular score in the group with a total reticular score of 5 or higher and defined as severe lung fibrosis is further reviewed (Figure 7, page 48).

**Figure 6. An overview of the localization of fibrosis in the lungs in those with minor (n=9), moderate (n=11) and severe fibrosis (n=24) based on the HRCT analysis.**



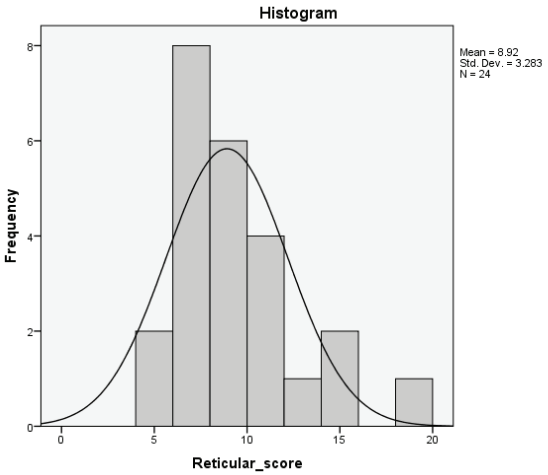



**Figure 7. The distribution of the total reticular scores in the group with severe fibrosis (n=24, total reticular score ≥ 5)**

There seems to be a subset of MCTD patients that develop more rapidly progressive lung fibrosis. This interpretation is supported by the finding of more severe lung fibrosis in patients with a shorter mean disease duration (6.4 years) compared to patients with minor or moderate fibrosis (13.2 years). Currently it is not clear why pulmonary disease, and particularly lung fibrosis, is so frequent in MCTD as this and earlier studies have shown. It could be related to the presence of anti-RNP autoantibodies, but the fact that anti-RNP positive SLE patients rarely develop pulmonary disease argues against this notion. Another possibility is that the MCTD subset who develop lung fibrosis have an SSc-like or PM/DM -like phenotype. This possibility is not directly supported by our clinical data, but it does appear that the reticular pattern seen in many of the MCTD patients to some degree resembles the pattern seen in SSc (188, 189).

In conclusion, the current study showed that HRCT findings compatible with lung fibrosis were frequent in Norwegian MCTD patients and the HRCT findings were associated with reduced pulmonary function tests, reduced overall physical capacity and increased mortality. Systematic follow-up studies of this nationwide Norwegian MCTD cohort will hopefully provide additional data on the long-term morbidity and mortality. The main goal of such study is to find and define markers and/or prognostic factors that can be used for early diagnosis and by defining patients at high risk of lung involvement and to select those for early treatment interventions.

**Esophageal involvement associated with lung fibrosis**

An esophageal involvement with gastroesophageal reflux (GERD) could be one of the pathogenic factors for lung fibrosis in MCTD (27, 190).

The grade of esophageal dilatation was estimated individually by two experienced chest radiologists (TMA & GKM) in the 126 patients with accessible HRCT files in paper II (2). A large collection of intraluminal air, fluid or air fluid levels, was classified as esophageal dilatation as well as visible distension of esophagus (27, 191, 192). Absence of esophageal dilatation was defined by less than six air bubbles and insignificant distension. Esophageal dilatation was defined by the presence of six or more air bubbles and/or distension (192).

There was a statistically significant association between the HRCT defined esophageal dilatation with both symptoms of GERD and earlier barium x-ray contrast investigations, in spite of many missed variables on barium x-ray contrast investigations (data not shown). Preliminary analysis supports an association between lung fibrosis (2) and esophageal dilatation (Table 8). A newly published systemic review on the relationship between ILD and GERD (193) in patients with and without CTDs identified 319 publications where 22 met the entry criteria, however without supporting an association between ILD and GERD. Limited data on MCTD seem, however, to support some association between GERD and ILD (27, 190).

**Table 8. Esophageal dilation evaluated from HRCT of the lungs associated with lung fibrosis as pathological reticular score.**

| | | Esophageal dilatation by HRCT | | |
|---|---|---|---|---|
| | | No | Yes | Total |
| Lung fibrosis | No | 64 | 18 | 82 |
| | Yes | 20 | 24 | 44 |
| | Total | 84 | 42 | 126 |

2-tailed p-value 0.0005 by the Fisher exact test

**Pulmonary hypertension**

The results of the third study (*paper III*) contradict most textbook statements (183), defining PH as a major disease complication in MCTD and occurring in at least one of four MCTD patients. This view is based on early data from three frequently cited small cohort studies (34-47 patients) from tertiary referral centers (7-9) and one larger screening study (20) solely based on echocardiography. PH appears to evolve both early and late in the course of MCTD and has a poor prognosis (7-9); the current study seems to confirm this in spite of the low number of PH patients.

All MCTD patients in the previous PH studies (7-9) were defined as having PAH, although a review of the data shows that many of them had severe ILD. According to the current classification of PH (117), some of these patients most likely had PH secondary to ILD, rather than PAH.

The 147 patients included in the present investigation were systematically screened for PH by echocardiography and those suspected of PH were referred for right side heart catheterization (RHC). The diagnosis of PH was according to the updated guidelines of the European Society of Cardiology and the European Respiratory Society from 2009 (124). After the initial PH screening, the patients were followed clinically for a mean 5.6 years. Data from HRCT and PFT were included to classify the PH, according to the updated 2008 Dana Point classification (117), as isolated pulmonary arterial hypertension (PAH) or as PH associated with interstitial lung disease (PH-ILD). The main finding was that the overall prevalence of PH in the cohort was 3.4% (5/147 patients), much lower than expected from previously published data. To ensure that PH diagnosis was not missed, all the 308 echocardiographs performed during follow-up were assessed and the causes of death in the 12 deceased patients were carefully reviewed. According to the United Kingdom National Registry data on PH associated with connective tissue disease, there were 36 cases with PH associated with MCTD diagnosed during a five year follow-up of the 60 million UK population (about 0.6 MCTD-PH per million over 5 years) (130). This gives a prevalence rate of MCTD-PH in the same range as the current Norwegian cohort with five MCTD-PH cases during a 5.6 year follow-up of the 4.9 million Norwegian population (1.0 MCTD-PH per million).

The main limitation of the current study is that the MCTD patients included were followed up according to the local department of rheumatology clinical practice but not by a standardized

protocol. That is, they were most often controlled by clinical assessment, often with annual PFTs (including $D_LCO$), HRCT and NT-proBNP measurements in plasma, a proven biomarker in PH which would minimalize the risk of missing PH patients. However, such assessments would not totally exclude the presence of mild and/or early PH.

Another potential weakness was that echocardiography, but not the gold standard RHC, was used as the primary screening tool for PH. The correlation between pulmonary pressures determined by echocardiography and by RHC is modest (127, 129), but by using a pulmonary artery systolic pressure (PASP) > 40 mmHg as a cut-off value for referring for RHC and estimating other echocardiography parameters combined with a clinical assessment.

There are no Norwegian or international guidelines regarding screening for PH in MCTD. Approximately two thirds (64%) of the patients in the cohort were examined by echocardiography at least once during follow-up. In spite of frequent echocardiography controls, the possibility of unnoticed cases of PH cannot be ruled out. A thorough review of the files of all the twelve deaths in the study, did not detect any undiagnosed PH.

The current study differs from the three other cohort studies. Firstly, and probably the most important factor was the selection of the study population. While three of the previous small (34-47 patients), cohort studies (7-9) assessed PH in MCTD cohorts from single center tertiary university hospital in USA. The current study selected MCTD patients from public hospital clinics in Norway and represents an unselected MTCD population. Patients followed at tertiary hospital care unit are generally sicker with more complicated diseases than those followed by others. Furthermore, MCTD case assignment was different in the current study compared to the earlier studies of which two studies used no MCTD criteria sets (7, 9) and the other (8), a single set (Kasukawa (5)), whereas the current study applied all three most common MCTD criteria sets (4-6). The present study only detected five PH patients, all of whom fulfilled both the criteria sets of Sharp's (6) and Kasukawa's (5), whereas four (4/5) fulfilled the Alarcón-Segovia criteria set (4), that does not include neither pulmonary nor cardiovascular manifestations.

Secondly, the female to male ratio was lower in the current study (3.3) and all patients were Caucasians. The three earlier studies (7-9) had female to male ratios higher than 10 and about 20% of the cohorts of Burdt's (8) and Sullivan's (9) studies were African Americans. PH seems to be more prevalent in women (194, 195) and African Americans appear to develop

PH more often than Caucasians (196, 197). Race and sex differences can probably explain only a small part of the PH prevalence rate differences.

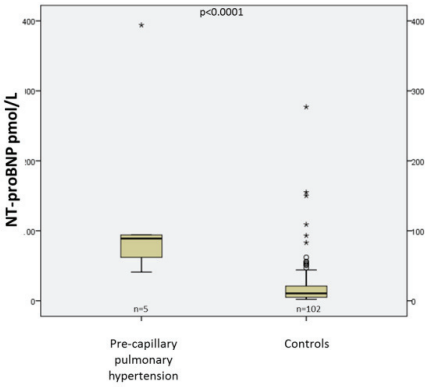
Thirdly, the mean follow-up time (5.6 years) in current study was shorter than the mean of 15 (8) and 6.3 years (9) in two of the previous studies. As two new PH cases were identified during follow-up, it cannot be ruled out that the frequency of PH may increase over time. At least some of the differences between the frequencies of PH in current study and one of the earlier studies (8) can be explained by the length of the follow-up time.

Finally, there is possible difference in the stringency of the PH diagnosis between the current and the previous studies. Here, PH was defined according to the RHC criteria set forth in the 2009 ESC/ERS Guidelines (124). Three of the previous studies also used RHC to define PH, but it is not clear if they also included patients who only had PH as defined by the now abandoned, exercise-related definition of PH (124). The fourth study defined PAH solely by Doppler echocardiography (20) where the definition of PH was based on a low estimated pulmonary artery systolic pressure of (PASP) ≥ 40 mmHg.

As reviewed there are serial differences in current study and earlier studies that can explain the differences in the prevalence rate.

An increased NT-proBNP level in plasma has been shown to have prognostic value in various forms of PH (120, 198). NT-proBNP is a natriuretic peptide and is mainly secreted by the ventricles in response to increase mechanical stretch and pressure (121, 122). The finding that the patients with PH associated with MCTD also had high NT-proBNP levels is in line with previous data from SSc (123) and was not surprising. In spite of the few PH patients this association was highly statistically significant and in fact the first time this association has been shown in MCTD-associated PH.

**Figure 8. N-Terminal Pro Brain Natriuretic Peptide (NT-proBNP) in blood.**




Differences between serum levels of NT-proBNP between those with and without pulmonary hypertension were tested by the exact independent sample Mann-Whitney U test, with two tailed 95% significance level. The difference is still highly satistical significant in spite of the outlier (*Patient 3, Paper III*) is removed from the analysis.

MCTD patients may have both ILD and pulmonary vascular disease as observed in SSc. It is uncertain to what extent PAH and PH-ILD represent two different phenotypes of PH. It can be difficult to establish whether the PH is an isolated vascular disease independent of the ILD or whether it is caused by the ILD. The third possibility is that both the PH and the ILD are secondary to pulmonary vasculopathy (136).

In summary, the current study suggested that the prevalence of PH in MCTD was substantially lower than expected from previous studies, but in spite of the few patients it seems to confirm the notion of seriousness of this disease complication.

# 6 Main conclusions

- The prevalence of MCTD was 3.8 per 100,000 and the incidence of adult onset MCTD was estimated to be 2.1 per million per year.
- MCTD had a female dominance with a female to male ratio of 3.3, without a statistically significant difference between the adult and juvenile onset disease.
- There was no statistically significant difference between point prevalence and incidence estimates of the three MCTD criteria sets used (4-6). However, different MCTD criteria sets seem to select different patient subsets regarding pulmonary disease.
- Lung involvement was common, with every other MCTD patient (52%) having abnormal high resolution computed tomography (HRCT) findings, most commonly reticular patterns consistent with lung fibrosis in more than one third (35%) and severe lung fibrosis in one fifth (19%) of the patients.
- Lung fibrosis in MCTD was uniformly concentrated in the lower parts of the lungs, similar to lung involvement in systemic sclerosis.
- Lung fibrosis was highly associated with reduced pulmonary function estimated with pulmonary functions test (PFTs), overall physical capacity and highly associated with increased mortality but not associated with smoking.
- The prevalence of pulmonary hypertension was lower than expected (3.4%).
- The association between a high value of the serum biomarker pro-BNP and PH in patients with MCTD was confirmed for the first time.

# 7 Clinical implications and future perspectives

## 7.1 Clinical implications

Findings from the three studies presented have confirmed that MCTD is an uncommon but in some cases a serious disease. The prevalence of PH in MCTD was lower than expected from several earlier small cohort studies (7-9) and that reported by several medical textbooks (183). In spite of the lower prevalence than expected (3.5%), the prevalence of PH was much higher than expected in a healthy population or in patients with other connective tissue diseases, except for SSc. There are currently no guidelines for screening for PH in MCTD such as there are for SSc (199). It is debatable whether it should be recommended to follow the current SSc guidelines for screening for PH with annual Doppler echocardiography also in all patients with MCTD given the relative low prevalence of PH found in current study. On the other hand Doppler echocardiography is a non-invasive study and PH is a life threatening disease complication where early diagnosis is important. With time it may be possible to select a subset of high-risk MCTD patients for PH screening.

The prevalence of ILD, especially lung fibrosis, was somewhat higher and the ILD was far more severe than expected as it clearly affected both morbidity and mortality. This would support a systematic screening for lung involvement in patients with MCTD and thus identify patients at risk and consequently lead to delaying and/or stopping progression of the lung fibrosis by early diagnosis and treatment.

## 7.2 Future perspectives

Currently, only a small part of the data gathered has been analyzed and published. There is ongoing work in analyzing data for further publications. One part of the work is to analyze the genetic aspects of the disease and possible associations to the phenotypic pattern. Another paper on pulmonary manifestations is planned, comparing the American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias from 2001 (144) with the latest Fleischner Society classification of ILD from 2008 (149). There are data on the

autoantibodies and on pregnancy and infertility that have not been looked at, in addition to other clinical data gathered.

Little is still known about MCTD. There are concrete plans to proceed with further studies on MCTD, partly based on follow-up of this nationwide cohort with another Norwegian nationwide screening, and to add newly diagnosed patients to the cohort. The aim is to evaluate the constancy of the MCTD diagnosis and find prognostic markers especially associated with the more serious aspects of the disease, the pulmonary and cardiovascular complications. It is important to evaluate the mortality and morbidity of the disease by following the current population longitudinally. Longitudinal follow-up will also give opportunity to identify and study further arthritis and myositis associated with MCTD as little is known of these disease manifestations, and finally to evaluate the prognostic value of capillaroscopy and genetics to MCTD phenotype and prognosis.

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# Appendix

## Papers I – III

# I

II

III